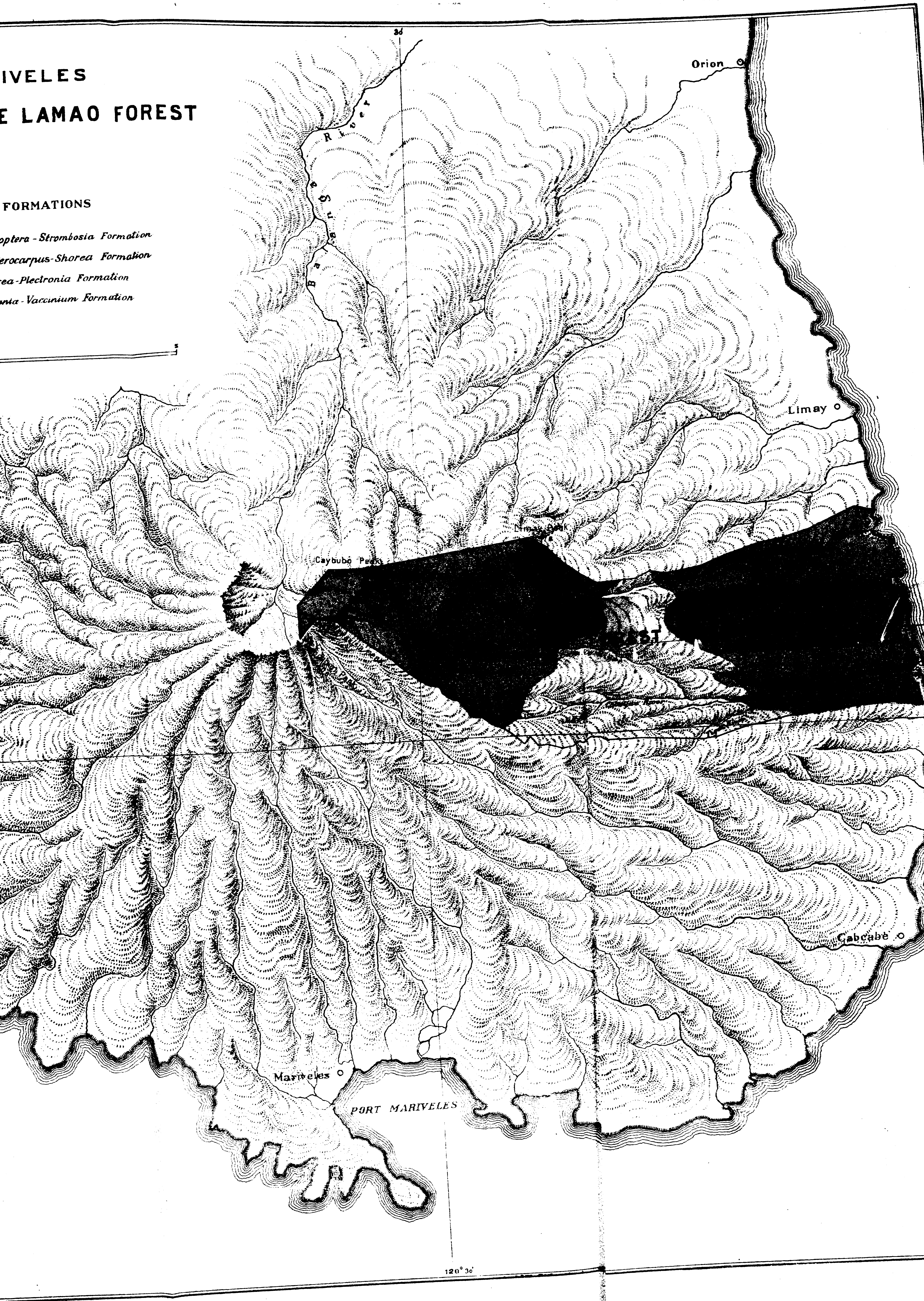


IVELES
E LAMAO FOREST
FORMATIONS
optera - Strombosia Formation
erocarpus-Shorea Formation
ea-Plectronia Formation
nia - Vaccinium Formation
Orion
Limay
Limay Peak
Caybubo Peak
Cabcabe
Mariveles
PORT MARIVELES
14°30
120°30

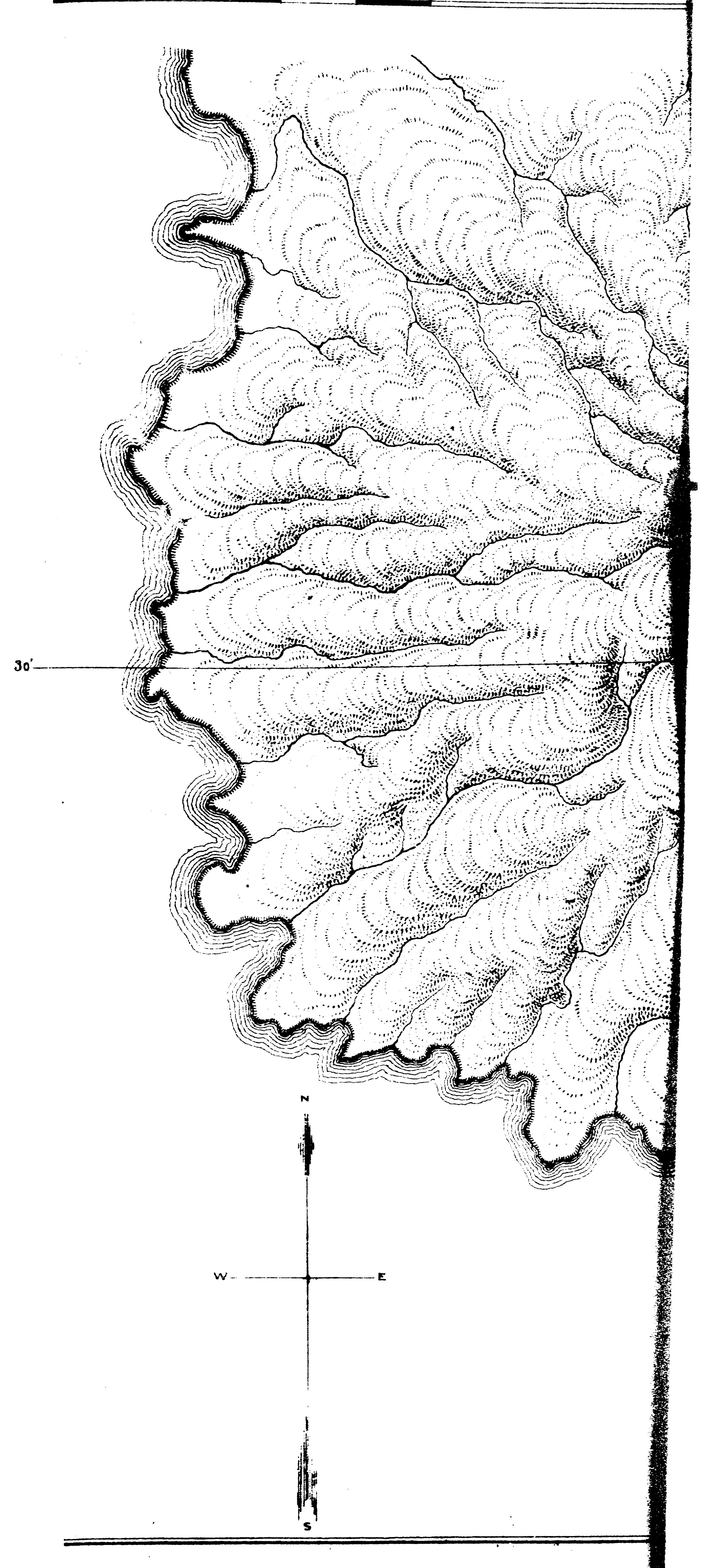
MAP OF MT. MARIVELES SHOWING LOCATION THE LAMAO RESERVE

LEGEND FOR PLANT FORMATIONS

- *Mangrove Formation*
- *Nipa-Acanthus Formation*
- *Barringtonia-Pandanus Formation*
- *Bambusa-Parkia Formation*
- *Anisoptera - Strombosia* [cut off]
- *Dipterocarpus-Shorea* [cut off]
- *Shorea-Plectronia Form*[cut off]
- *Eugenia - Vaccinium Form*[cut off]

Kilometer

5 4 3 2 1 0



THE PHILIPPINE

JOURNAL OF SCIENCE

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THE PHILIPPINE JOURNAL OF SCIENCE.

The Bureau of Government Laboratories of the Philippine Islands, after an existence of four years under that title, has been enlarged in its scope by the addition of the former Bureau of Mines, and in order to emphasize this general increase in its functions the name has been changed to that of the Bureau of Science. As was the case with the former Bureau, the new one will conduct investigations on general chemical and biological problems involving bacteriology and immunity, the production, improvement, and standardization of sera and prophylactics, pathology, the etiology, treatment, and prophylaxis of tropical diseases, botany, entomology, and a study of the avifauna of the Islands; chemical questions relating to the natural products and resources of the Philippines and other subjects which come within the scope of a collection of laboratories. In the future, owing to the consolidation with the Bureau of Mines, the Bureau of Science will also undertake work in geology, paleontology, mineralogy, and on mines and mining engineering.

In the past, the research work of the Bureau has appeared in a series of bulletins, thirty-six in number, the individual ones of which have been distributed to the public on demand. In altering the scope of the institution it seemed expedient to change the form in which the work should appear. Therefore, the Bureau of Science has decided to publish its researches in a journal to be conducted under the auspices of the Bureau. This periodical will contain original articles by members of the Bureau staff as well as by others who, in the Philippine Islands or adjacent countries of the Orient, are doing scientific work of the proper character. The JOURNAL will thus have the unique function of expressing the united scientific results achieved in contiguous countries situated in the Tropics. With the growing interest which is being taken not only in tropical medicine but in the agricultural and mineral resources of these regions, it seems certain that a publication of the character which is proposed will

secure a large number of readers. In the beginning it would not be expedient to put out the JOURNAL in sections devoted to individual realms of science, it being better to unite all phases of work in one periodical. At a later date, when the number of investigators in the Islands will have increased and when the contributions from outside sources will be of sufficient number, the JOURNAL can be divided into proper sections, each one of which will be issued as a separate volume.

Considering the interest which has been manifested in the past in the bulletins of the Bureau of Government Laboratories and the certainty that the same, or even a higher, standard of work will be maintained in the future, the permanency of THE PHILIPPINE JOURNAL OF SCIENCE is assured, and while individual readers may find papers in it which have no connection with their particular specialty, in the course of a year it is certain that one or more important researches on each line of investigations will be published.

PAUL C. FREER,
Director of the Bureau of Science.

ON THE WATER RELATIONS OF THE COCONUT PALM (COCOS NUCIFERA)—ON THE OIL PRODUCED FROM THE NUTS—THE FACTORS ENTERING INTO THE RANCIDITY OF THE OIL, AND THE INSECTS ATTACKING THE TREES.

Introduction by PAUL C. FREER.

Investigations on the subject of the coconut palm (*Cocos nucifera*) have been carried on in the Bureau of Government Laboratories for the past eighteen months. The work has been divided into three parts and brought to its present state by coöperation between several divisions of the institution. It will be published in serial form in the JOURNAL. The first portion covers the water relations of the tree from the standpoint of its physiology, by Dr. Edwin Bingham Copeland, who spent several months on a plantation studying this question from an experimental standpoint. The second paper covers the coconut in its relation to the cultivation of the tree and the production of coconut oil, and includes a study of the deterioration both of the copra and the oil by reason of rancidity caused by molds and bacterial growth, by Herbert S. Walker; and in conclusion there is added a study of the insects which attack the plant, together with suggestions as to the best means of combating their depredations, by Charles S. Banks and William Schultze.

By this union of the laboratory work, the study of this most important tropical tree has been carried to an extent which not only will enable the conclusions to be of great value to planters but which will also have a scientific interest for those who are not immediately interested in coconut production. One topic which is of especial importance is still under investigation and not ready for publication. This is the study of the germinating nut together with the transformation which the oil undergoes during the growth of the embryo. This topic offers an opportunity for the study of the enzymes in a germinating plant which is unsurpassed, as the size of the seed of the coconut and the ease with which it is separated into its various constituent parts brings a certainty of results not to be encountered in other instances. This portion of the investigation is now being followed in the chemical laboratory. When the serial on the subjects mentioned above has been completed it will be published as a separate reprint.

San Ramon Government Farm, where most of these investigations were carried on, lies on the west coast of Mindanao 10 miles north of the town of Zamboanga. It extends for about 2 miles along the seacoast and toward the interior for 3 or 4 miles, to the base of a small range of densely wooded mountains, which forms an admirable watershed.

Four small streams run through San Ramon from the mountains to the sea. It is very probable that there is considerable underground drainage as well, for fresh water may be obtained at a depth of 5 or 6 feet almost anywhere along the shore, even at the edge of the beach. At present copra and hemp are the principal products of the farm, together with a little cacao.

At the time of writing all the coconut trees used for making copra at San Ramon were planted by the Spanish, but large numbers of new ones are being set every year from selected seed, for which only the largest and best nuts are taken. They are laid out on the ground in a sheltered place and a small section of husk is cut from the top of each to afford a more easy egress for the sprout. At the end of about six months' time, when the sprout is from 2 to 3 feet high and the nut has just begun to take root in the ground, it is ready for planting. For this purpose a hole about 2 feet deep is prepared and the young plant is firmly packed with the soil, so that the sprout stands erect and the top of the nut is 6 to 10 inches below the surface. As a protection against wild hogs it has of late been the custom to dig a pit 4 or 5 feet deep and to plant the nuts at the bottom of this. The seedlings are set out in straight rows, allowing a space of about 10 meters between each plant.

After planting, the young coconut requires very little care, except to keep it free from weeds and the attacks of animals and insects, until it reaches maturity. The average time before a tree begins to give a good yield of fruit may be set at ten years. Instances have been known when bearing commenced as early as the fifth year, but these are of rare occurrences and under exceptionally favorable circumstances.

The process in use for preparing copra is very simple. The nuts are gathered by natives, who climb the trees, cut off the ripe or nearly ripe fruit, and let it fall to the ground. No especial care is taken to prevent damage by falling. The nuts are then piled in a heap and allowed to stand for a few weeks before being opened. To remove the outer, fibrous husk the natives make use of a heavy spearhead firmly sunk in the ground. They force the nut down on the sharp point until it penetrates to the shell, then, by a peculiar twist, strip off the husk, a portion at a time. One man can husk, on an average, 1,000 nuts per day.

After being thus prepared the coconuts are split in halves by a couple of sharp blows from the back of a bolo. The milk is allowed to go to waste on the ground.

Drying.—The simplest method of drying the meat is to spread out the halves of the coconut on large wooden trays, face up, in the sun. At

night and in case of rain the trays are piled under a shed. After standing in the sun for two or three days the meat becomes partially dry and has shrunken sufficiently to permit its removal from the shell. It is then put back on the trays and again exposed for a few days until it is thoroughly desiccated.

The other method of preparing copra in use at San Ramon is to pile the coconut halves, face downward, on a bamboo grating over a slow fire of husks which is burning in a thick-walled brick kiln about 6 feet high, the whole being inclosed in a large shed. By this arrangement it is sufficient to dry the nuts over night before removing the shells.

After heating the meat in the same manner during four or five hours on the next day, it is ready to store for the market. "Grill-dried" copra prepared in this way is not quite so liable to be attacked by insects and molds, but on account of its dark color and slightly smoky flavor it is considered inferior in quality to the sun-dried article.

ON THE WATER RELATIONS OF THE COCONUT PALM (COCOS NUCIFERA).

By Edwin Bingham Copeland.
(From the Botanical Section of the Biological Laboratory, Bureau of Science.)

The work on *Cocos nucifera* (coconut), the result of which is reported below, was performed at the Government farm at San Ramon, near Zamboanga. Its purpose was to acquire as thorough a knowledge of the physiology of this palm as the field conditions would permit, with the especial hope that the results would be available for improving existing methods of the plant's cultivation.

Because of the remoteness of the place of work from any library or base of supplies, the simplicity of apparatus which for the greater part is used in investigating all phases of a plant's transpiration, the writer's familiarity with this particular field, obtained in the preparation of earlier papers, and because of the very great practical importance of understanding this phase of the physiology of any plant important in agriculture, the work was principally focused on the water relations of the coconut. At the same time other phases of the tree's activity were not neglected; and, in cases where it seemed worth while, notes not bearing on the main subject are included in this paper. The value of artificial or natural fertilizers was not considered, because this question is more in the domain of the agriculturalist.

The divisions of the main subject are treated in the following order: The root—its structure and growth, and the absorption of water; the leaf—its structure, the activity of the stomata, and the transpiration; with final conclusions as to the fitness of the plant for its characteristic habitat and suggestions as to its most advantageous cultivation.

THE ROOT.

The roots of *Cocos nucifera* have the two typical root functions—the anchoring of the tree and the absorption of the water and mineral food necessary for its maintenance and growth. In the absence of a taproot, or of any great roots the hold of which in the ground can maintain the rigidity of the trunk, the mechanical problem of the firm anchorage of the latter finds a solution essentially different from that which we are accustomed to encounter in the case of dicotyledonous trees. The base of

the trunk is convex or obconical, and is usually buried for a depth of hardly more than 50 centimeters. Its surface underground is almost entirely covered with the bases of the roots. The latter are remarkably uniform, about 1 centimeter in diameter, radiating from the tree on all sides, each without much variation in its direction, and, so far as my observations justify a general conclusion, for a normal distance of about 5 meters in firm soil and 7 meters in sand. The lateral branches, whatever direction they may take with regard to the action of gravity, leave the main roots with surprisingly uniform exactness at right angles and are likewise on the whole straight, though less so in detail than the main roots.

The old main roots are notable for the combination of elasticity [1] and tensile strength shown by their powerful central steles, the cylinder of xylem inclosing a "pith" with thick, lignified walls. The most conspicuous feature of the branches is their stiffness, for which the stele is not more responsible than the hypodermis. I have never before, in any plant, seen a rigidity on the part of the fine, absorbing roots which will compare with that possessed by those of the coconut. The intimate contact between the hard, firm roots and the soil is responsible for the rigidity of the *Cocos*, as of other trees, but while in most, this contact is centered about the base of the trunk, the *Cocos* has it disseminated equally through the ground to a radius of 5 meters or more. The main roots act as so many taut strands between the base of the trunk and the multitude of fine points of attachment. The effectiveness of the coconut's system of anchorage is perfect. The tree's favorite habitat is the seashore, where it receives the unbroken force of the fiercest storms. Because of its elasticity, the trunk very rarely breaks, and I have never seen one instance of an uprooted coconut, the roots of which had not either previously been killed or undermined by waves.

Eighty centimeters is not a very exceptional diameter for a well-grown bole, though a majority fall below this size. The buried part of a stem of this thickness will afford attachment for nearly 8,000 bases of roots 1 centimeter in diameter. Some of the main roots bear few or no branches at all like themselves; others have 10 to 20, which rarely reach a length of 1 meter or a diameter of 4 millimeters. The main roots and these major branches bear numerous fine ones, 1 to 2 millimeters in diameter, springing forth at right angles and having a rigidity which has already been noted. These may be the ultimate divisions; or they in turn may bear finer branches, at most a very few centimeters long, about 0.5 millimeter in diameter; the life of the latter is transitory like that of root hairs. A less ample system of branches is formed in sand than in firmer ground.

Dead, distal parts of roots are replaced from the bases of the same roots

[1] Pfeffer, *Pflanzenphysiologie*, II, page 60, cites Sonntag, *Landw. Jahrb.* (1892), **21**, 839 as authority for a stretching of 20 per cent by *Cocos* fibers, without exceeding their limit of elasticity.

in such a way that the new takes the place of the old, not only as an absorbing organ but in the mechanical system as well. At the point where dying back ceases, a root, or frequently two roots, spring from the end of the part which is still living. Their origin apparently is internal, and, as is to be expected, from the outer limit of the stele; the hypodermis of these older parts of old roots is so strong that the young ones are rarely able to break through it, with the result that they grow onward within the shell, sometimes for 30 centimeters or more, before the hypodermis is sufficiently decomposed to permit their escape. The direction of growth is then well fixed. From an observation of exceptional cases in which the young root succeeded in rupturing the hypodermis at its origin, and in which it then grew along or near it, it appears that as a phenomenon of "correlation" the young root has the same orientation-reaction as the one it replaces. The old hypodermal shell is a most effective aid in this reaction.

My observations on the rapidity of the growth of roots have been unsatisfactory. Many times I have marked off zones on apparently healthy roots only to discover that they showed no subsequent growth. Some, for a time, have elongated little or not at all, then for a few days have grown vigorously, then stopped, without any apparent reason for the irregularity:

The most rapid growth I have measured was 3.5 millimeters per diem. In a month three roots grew more than 4 centimeters, but none as much as 5 centimeters. Sometimes, under favorable conditions, there may be a much more rapid growth than I have been able to observe; 3.5 millimeters per diem is hardly more than 1 meter per annum, a rate too slow to be accepted without more evidence. A part of the roots I examined grew in water and a part in air surrounded by soil. Those which elongated considerably in water at the same time became more slender.

In large and rapidly growing roots a little elongation occurs in a zone 10 to 15 millimeters from the tip (not from the growing point), but in most cases it is confined to the apical 10 millimeters. The root whose growth was most rapid was 9 millimeters in diameter and had a cap 10 to 11 millimeters long. In two days the latter grew 0.5 millimeter, 5 millimeters of root grew out of it, and the zone immediately outside grew 1.5 millimeters. The length of the cap is somewhat greater than the diameter of the root, which is usually about equal to the length of the growing zone when measured from the outside tip; therefore all growth is generally within the cap. In this case the cap grew one tenth as rapidly as the root, and this seems to be about the usual ratio. In the ground the resistance to the passage of the moving tip results in a continual tearing off of the outer layers of the cap, these layers usually persist in the form of collars around the root, and each is about as long as the cap; altogether they not infrequently form a sheath along the whole younger part of the root. It is possible that these collars or sheaths facilitate the absorption of water. When the root grows without friction, in water, the whole outer portion of the cap, while retaining its form, is occasionally sloughed off.

No response to any other directive agent is so conspicuous as the autotropism of the coconut roots, of whatever order. The general level of the main roots is maintained by a combination of hydrotropism and aërotropism, which I have not

been able to analyze. A recent paper by Bennett[2] shows that many cases, at least, of apparent aërotropism are really hydrotropic, and the same is probably true with the *Cocos*. The roots maintain a deeper level in sand than in heavy soil. When the other stimuli are removed, a variable geotropism shows itself; some, in water, grow straight ahead in as nearly horizontal a direction as it was convenient to arrange them in the bottle; but the majority show a feeble positive geotropism, the most rapidly executed curve being 40° in two days. The secondary roots are usually controlled by their autotropism alone. In heavy soil they are sometimes more numerous on the upper sides of the main roots, probably because of an induced geoauxesis, since the structure of the roots precludes the probability of any direct locative influence of moisture on their origin, and the pneumathode roots appear on all sides. In nature, no roots will grow to any distance into water, nor into a level of the soil where water stands; and a rise in the water level ultimately kills the submerged ones.

Root structures.—The stelar tissues of the coconut root offer very that needs description. The number of xylem rays is usually 40 or more in the larger, 10 to 15 in the branch roots, 1 to 1.5 millimeters in diameter, and fewer in the finer ones. In the young parts of the main ones the pith is parenchymatous, with very thin walls. The latter begin to thicken at a distance from the apex at which both hypodermis and endodermis have reached their permanent state. They then become very thick throughout, and are the chief source of the root's great tensile strength.

In cross sections, a very few cells behind the growing point, the pericycle is distinguishable by the regularity and the large size of its cells. The latter eventually become somewhat flattened tangentially, but they still form a conspicuous layer in sections of old roots, as their walls remain thin and colorless. The cross partitions are reticulate-punctured.

In very young parts of the root the endodermis can be identified only by reference to the pericycle (fig. 2). Its cells begin to thicken at about the same point as do those of the hypodermis, where the latter begins to interfere with the absorption of water. The thickening takes place cell by cell, rather abruptly in the individual cells, but without any uniformity throughout the layer, so that in some sections a few will be found well thickened, all the others still thin; while a little farther back most of them will be found to be thick. Counting all the endodermal cells in a section, an undue proportion of those which thicken late is directly outside the xylem rays, where passage cells would be expected. However, cells in this position are not infrequently among the first to thicken, whereas scattered ones found elsewhere are often among the last. Considering the zone with reference to the hypodermis at which the thickening of the endodermis begins, it is evident that it is only as the water travels obliquely up the root to the stele, and not directly inward, that any of the cells remaining thin have occasion to serve in its passage. The appearance of the old endodermis is shown in the accompanying figure (fig. 1).

[2] *Bot. Gaz.* (1904), **37**, 241.

The outer part of the cortex, immediately underlying the epidermis to a depth of three to six layers, is composed of cells smaller than the deeper-lying ones. The walls of these, while they are in young and active parts of the roots, are very thin, with a notably dense protoplasm. Subsequently the walls thicken, those of the outermost cells first, until the lumen is almost obliterated; they acquire a stony hardness and dark color (figs. 3 and 4), thus forming a closed shell around the root, protecting it against animals or fungi and having a mechanical value already mentioned. The imperviousness of this shell to water is shown by its effect on the epidermis and on the formation of pneumathodes. The zone in which the hypodermis forms is that at which the root ceases to absorb water from the soil.

The larger the root, the farther from the tip is this likely to be. In very active ones the distance is as much as 5 centimeters; in those less active, but by no means inert, having a diameter of 7.5 millimeters, it is found to be 2 centimeters from the tip, while during drought it advances to a position well within the firmly adherent part of the cap.

Between the hypodermis and endodermis the cortex is composed of rather large cells, isodiametric or somewhat elongate longitudinally, with thin, colorless walls, watery contents, and numerous intercellular spaces (fig. 5). After the layers bounding it reach their final state, parts of the interlying cortex become unequally thick walled and lignified. At the basal end of old roots this intermediate cortex breaks, probably as a result of tension between the elastic stele and nonelastic shell, leaving the former loose inside of the latter.

The dermatogen is questionably distinguishable around the growing point, even in most favorable sections. The epidermis is a transitory tissue, dying when its connection with the inner part of the root is interrupted by the development of the hypodermis. Its most conspicuous feature is that the least diameter of its cells is the longitudinal (figs. 6–8). No root hairs are ever formed, but the superficial area is slightly increased by the breaking apart of the outer ends of the cells—a process which is most evident in longitudinal sections. In a soil where the supply of water is even moderately constant and ample the coconut root, with its short absorbing zone and absence of hairs, would be regarded as but a poor water gatherer, but when water is abundant, hairs are not needed; and in a dry time their sacrifice is spared to the coconut. A tree whose normal economy is planned on the absence of root hairs is comparatively well able to survive periods of abnormal difficulty in obtaining water.

Pneumathodes (figs. 9–14).—The development of the hypodermal shell so completely cuts off the interior of the root from all communication with the outside that it can not carry on the limited exchange of gases necessary for its respiration, and therefore it is obliged to develop special breathing organs, or "pneumathodes." These are specialized roots which quickly grow to a length of from 3 to 6 millimeters and then abruptly

stop. The cells of the cortex then enlarge; at first they keep their form, but afterwards they become spherical and finally put out processes each of which keeps in contact with a corresponding one from an adjacent cell. This enlargement of the cortex ruptures the epidermis and the growth of the inner layers separates the outer ones, so that the epidermis and outer layers flare back from both ends of the swollen zone; its surface is then mealy in appearance and white because of the contained air. As the pneumathode ages, the cap and all the outer tissues beyond the open zone slough off; the strongly lignified stele gives it stability and its sharp point will protect it against mechanical injury, if protection is needed. The cells of the open tissue necessarily promptly die, but their walls remain firm, their surfaces become granular, and in this condition they can not be wetted, so that the large amount of air contained between them can not be displaced. The cells next to the stele, and those at the base of the pneumathode—that is, those toward the parent root—enlarge moderately and become spherical, and thus form intercellular spaces of some size; their surfaces also become granular and their walls very thick, thus insuring the permanency of open aërial communication through the pneumathode to the tissue of the parent root, which has the most abundant system of intercellular spaces—that is, the cortical parenchyma.

Roots which have suffered metamorphosis to serve as pneumathodes have been encountered in many plants, and have been most thoroughly studied in this part of the world,[3] but in all previously known cases they are formed as a response to the wetness of the environment. In many plants which grow in wet places, either frequently or invariably, pneumathodes have become normal structures; in many others, whose roots only exceptionally find themselves where the supply of air is cut off by water, pneumathode-like structures form as abnormalities.[4] In plants whose habitat is such that pneumathodes have become a normal structure, the roots which serve this purpose have usually acquired a negative geotropism, adapting themselves to the direction in which the air is to be found. This is true of Phoenix, whose pneumathodes, as figured by Tischler,[5] are very similar to those of *Cocos*.

In distinction to all other known pneumathodes, those of *Cocos* are demanded by the structure of the plant without regard to what its environment may be. They form on roots in water, in firm ground, in loose sand, and in the air. In soil containing free air, where the roots normally grow and the formation of the pneumathodes is under the control of

[3] Karsten: Ueber die Mangrove-Vegetation im malayischen Archipel. *Bibliotheca botanica* (1891), Heft 22.

[4] The same is true of other parts of the plant as well. See Sorauer: "Ueber Intumescenzen." *Ber. bot. Gesell.* (1891), **17**, 456, and my note on Haberlandt's new organ on Conocephalus. *Bot. Gaz.* (1902), **33**, 300.

[5] Tischler, G.: Ueber das Vorkommen von Statolithen bei wenig oder gar nicht geotropischen Wurzeln. *Flora* (1905), **94**, 35.

natural selection, it is of course a matter of indifference whether they are above or below the parent roots; and no factor of the environment has the least influence in determining the place of their origin or the direction of their growth. They spring out at right angles, in all directions, and are straight. In water and in air they behave in exactly the same way. Exceptional length and negative geotropism would be appropriate reactions on the part of the pneumathodes emerging under water, but since the roots will not grow into water nor into soil without free air, their formation in this situation must be too abnormal and too rare a mischance for natural selection to have evolved any adaptation to it.

Absorption.—The same forces operate to draw water into the roots of plants which afterwards cause its movement to the leaves. There are—

(1) Suction exerted by the tissues surrounding the xylem ends in the leaves, and ultimately due to evaporation from the leaves under the influence of the sun's radiated energy.

(2) The osmotic activity of the cells in the roots through which the water passes. The former is the major factor, and its dominance is more extreme in the coconut than in most plants. This is clearly shown by two facts, the first one being that dead tips of roots for some time continue to absorb water without any measurable decrease in the rate as compared with that which was present while they were alive, and the second one is that if the tips of active, growing roots are cut off and immersed in water with not more than 5 millimeters of the cut end emerging into a saturated atmosphere, drops of water are not exuded from the cut surface; it merely remains damp. When roots are cut or broken in the ground, a gummy substance with a characteristic odor sometimes exudes, but there is neither bleeding of water nor of a dilute solution.

However, water entering the roots through the living epidermis and passing through living cells of the cortex to the stele must move under the immediate influence of the osmotic activity of these cells; a movement of the water under natural conditions is thus effected because it is constantly withdrawn from the inmost layers by suction. In this way the turgor of the roots is a factor in the acquisition of water, even in those which never bleed. The absence of bleeding only demonstrates that the living cells of the root will not pass a part of their osmotically active substance along with the water to the xylem; high turgor in the roots and abundant water in the soil will not necessarily result in root pressure.

The turgor in the pith, and in all except the fine outer cells of the cortex of the absorbing zone of the roots, equals 0.25 to 0.3 normal potassium nitrate solution. The walls are so thin that they wrinkle everywhere when plasmolysis is extreme (fig. 5). In the fine cells, which later become the hypodermis, plasmolysis is not visible in a less concentration than 0.5 normal; it is possible that the denseness of the protoplasm, together with the osmotic pressure caused by the cell sap, is responsible for this rather high figure. Plasmolysis is hard to detect in the epidermis. The turgor usually, but not always, seems to be a shade higher

than it is in the most of the cortex—about 0.3 normal. In the roots of most plants there is a slight but not appreciably interrupted increase in the turgor, from the epidermis inward; but this increase is no necessary condition for the ready movement of water, and in the *Cocos* we find in practice the lower turgor to be internal.

In the youngest cells of the embryonal tissue which can be plasmolysed the limit is 0.5 normal. In the cap the turgor is for the most part 0.25 normal; and in outside cells, as long as they are alive, it is no less. All these determinations were made on roots which were apparently healthy and active.

In all my experiments on absorption by the roots homeopathic vials were used, of such a size that when filled to the proper point with water the weight was 40–45 grams. In the cork of each was cut a hole fitting the individual root to be used. The latter was freed from the ground, with the least possible damage, to such an extent as to permit the necessary downward inclination of the tip. It was then washed, and all loose remains of the cap were carefully removed from the part which was to be within the bottle. To insure the absence of any open wounds the whole exposed part of the root, except that which was to be within the bottle, was smeared with vaseline. Water enough was used to immerse more than the absorbing region of the root, and the bottom of the bottle was kept low enough to prevent the water from touching the cork. The root, with its bottle, was laid in half of a split joint of bamboo, to which the appropriate slope was given, and the other half of the joint closed over it, thus insuring cleanliness. The hole in the ground was covered with abacá leaves to prevent unnatural warmth. All roots were left in this condition for one or more days before determinations of weight began.

After this time, when any initial disturbance in the rate of absorption was assumed to have passed, the hole and bamboo were opened, the bottle carefully removed, the root being touched by the bottle once to remove any free drops, and then a weighed bottle of water was substituted, the cork always remaining with the root. When all necessary care was taken to prevent wetting the cork, neither bottle needed to be open for more than five seconds, and the exposure of the root was even for a shorter time. The chief error in this method of experimentation is probably to be found in the variable amount of water adhering to the root, but experience shows that the results are reliable to a limit of 1 centigram.

The chief facts I endeavored to ascertain with regard to the absorption by the roots were the rate at which it normally takes place and the regular diurnal variation, if any, which may be found in this rate. I have also made some experiments on the absorption of solutions of potassium nitrate.

With regard to the usual rate of absorption, as has been seen to obtain for the growth, the first preliminary series of experiments demonstrated that roots which to the eye appeared to be similar behaved very differently. Nor was there correlation between vigorous growth and rapid absorption.

I made four sets of experiments, with essentially the same results; here it will suffice to give one of these.

This set was begun on January 11 and weighing commenced on January 13; but this beginning was made abortive by a rain which flooded the whole site. A new start was made on January 17. The weights given here are in centigrams, and are the average absorption for each day comprised in the interval ending at the date at the top of the column:

Root No.	Jan. 20.	Jan. 23.	Jan. 27.	Jan. 31.	Feb. 3.	Feb. 10.	Mar. 1.
I	6	6	6	7	7	3	7
II	15	6	15	19	19	14	12
III	19	8	[a]6	11	14	[b]22	28
IV	8	8	2	3	9	8	7
V	[a]16	18	28	35	43	43	(3)
VI	7	11	10	[b]3	3	3	.6
VII	[a]48	[c]47	52	53	40	[d]39	39
VIII	6	7	8	9	[d]5	7	8
IX	3	2	6	6	[c]5	7	------
X	41	33	[c]39	39	49	36	17

[a] Pneumathodes appeared.
[b] Growth conspicuously rapid.
[c] Apparently dead or dying.
[d] Cap sloughed (absorption greater than figures show).

The root V was injured February 10, and was then cut with a sharp knife without exposing the surface to the air, and the cut surface was then immersed just as the uninjured tip had previously been; the total subsequent absorption was only 63 centigrams. I had already satisfied myself that practically no water can be absorbed by cut leaves, and the same disadvantage from the experimenter's standpoint is presented by the roots. It is of interest to note that while an open wound is very promptly plugged, dead tips maintain their full absorbing activity for a considerable length of time.

From these results I do not believe accurate conclusions can be drawn as to the total absorption by an entire tree. The very great diversity in the rapidity of absorption by the roots is but one of the reasons for this. From a considerable number of measurements on different roots I can say that, as a general average, the end of a main root, which, on anatomical grounds, appears to be in a condition to absorb water, has about one-sixth of the total surface possessed by all the root tips tributary to it. If absorption were proportional to the exposure of living epidermis, then the most rapid rate exhibited by any of these roots would indicate a total daily absorption by a large tree of only about 24 liters. But there is no such correlation between living epidermis and absorption, as is shown by the behavior of dead roots and by the two mentioned in the preceding table, the growth of which was temporarily most conspicuously rapid. The immediate result of the rapid growth was a long zone of young tissue, but in one of these cases the ensuing absorption was remarkably slow.

The tips of the fine ultimate branches do not individually absorb with sufficient rapidity to give me trustworthy differences in weight, and they are too far apart to permit the use of several at once without a disproportionate increase in the water to be weighed. In a single instance I was able to include three of them in one bottle of the usual size, and then the observed absorption per unit of area was about three times as great as I ever found it with the tips of the main roots. No far-reaching conclusions are to be based on one fortunate observation; but it does show, as we must also conclude from the experiments to be described on transpiration, that the total absorption can be much greater than measurements made even on many tips of main roots would indicate. In one experiment, the tip of a small main root 5.5 millimeters in diameter showed a maximum rate for the time covered by eight weighings of 2 centigrams per diem.

Because of the slight difference in weight to be determined, it was useless, in undertaking experiments to show the relative absorption during different parts of the day, to work with roots which had not already shown themselves to be among the most active. In two sets of experiments I have used such roots for this purpose. The result has always been that the greatest relative absorption was observed during the afternoon, and, so far as any conclusion could be drawn in such detail, during the latter part of the afternoon. This difference, at different hours, is usually less marked than it appears to be from the following table, which shows the results for one day with the four most active roots represented in the preceding table. The roots bear the same numbers. This experiment began at 6.15 a. m. February 1. The figures are centigrams of water absorbed during the preceding interval:

Root No.	Feb. 1. 12.15 p. m.	Feb. 1. 6.15 p. m.	Feb. 2, 6.15 a. m.
II	5	11	5
V	14	21	16
VII	10	19	10
X	12	22	17

From the fact that decidedly the most rapid absorption is during and closely following the hours of most rapid transpiration, it is a reasonable conclusion that the tree contains practically no store of water on which it can easily and safely draw. However, no conclusion is justified as to the total water actually contained in the path of the transpiration stream, and therefore none as to the rapidity with which the water moves. The water may rise slowly but the demand still be propagated rapidly.

My experiments on the absorption of potassium nitrate are open to the same criticism as pertains to all of my other absorption experiments,

namely, they had to be made on the tips of the main roots, which are not the places where the process is most active. In working with these solutions, trouble with red ants, which only exceptionally interfered with experiments with pure water, became serious; as a consequence I was finally obliged to seal all the tops of the bottle deeply with vaseline, thus completely cutting off the access of air to the water in the bottle; controls with pure water showed that during the time of these experiments very little if any interference with the absorption resulted. The investigations were made with the same roots which furnished the material for the preceding tables, and they immediately followed the conclusion of the period already reported. These results were scattered through too many days to make a tabulated report feasible. In each case the absorption of the solution is compared with that of water during the preceding period, which usually was of one day.

A solution of 0.1 normal reduced the rate of absorption for root VII from 40 centigrams (for the preceding twenty days) to 35 centigrams, which is within the limits of daily fluctuation. It was likewise questionable, in the case of main roots, whether there was any reduction by a 0.2 normal solution; for instance, with root II the rate actually increased from 14 centigrams to 15 centigrams. However, in the case of the three lateral roots, the rate fell from 51 centigrams to 16 centigrams, and after two days they were evidently unsound.

The results obtained with 0.5 normal solutions were various. With root III the decrease in absorption was only from 18 centigrams to 8 centigrams; tested again with water, the rate rose to only 10 centigrams; another application of the solution reduced it to 7 centigrams; and in water it again rose to 10 centigrams. With root I, the previous rate having been 7 centigrams, successive determinations were 1 centigram, 1 centigram, and 2 centigrams; in water the rate returned to 8 centigrams. With other roots the half-normal solution was found to be sufficient to reverse the movement. Thus root VI, which had been very regularly absorbing about 1 centigram, *lost* 2 centigrams, 3 centigrams, and 2 centigrams. Root VII lost 1 centigram at one time and the three fine roots lost at the same rate.

Immediately after losing at the rate of 1 centigram for four days, root VII was put into a normal solution, and it then gained 8 centigrams in one day. This result, which at first sight was surprising, is easily explained. Water moves through the root in the direction in which it is driven by the greatest pressure. Under ordinary circumstances this direction is inward because of the influence of the atmospheric pressure, the pressure within being less than that without. This may be expressed by stating that there is a "suction" from the inside. In using the more dilute solutions other agents must have acted together with the atmospheric pressure—agents which perhaps were put in operation by the solutions themselves; in this way the fact that the solution is absorbed will account for the result. With the half-normal solution the osmotic pressure was superior to the sum of the forces tending to make the water enter; as a result, it moved outward. Other roots may have absorbed this 0.5 normal

solution more readily, and so have been able to keep up a slow, inward flow, for in proportion as it is absorbed it exerts no pressure. But the normal solution was sufficiently concentrated to plasmolyse all the living cells, after which it was possible for the solution to travel from outside of the root into the xylem without being compelled to pass through any of these. When this condition results there is no semipermeable membrane in its way, and, concentrated as it is, it can exert no osmotic pressure. If the half-normal solution were to cause general plasmolysis then it also would enter freely and for the same reason.

The turgor of root VI was tested. This root had lost water to the half-normal solution. A few cells in its cortical parenchyma were found to plasmolyse in this solution, but the turgor of most of them was decidedly higher—about 0.7 normal. Some cells which did not plasmolyse even in such a solution did so in a normal one. There was no active epidermis, for the hypodermis had developed so as to be only 1.4 millimeters behind the growing point, well within the adherent part of the cap. The turgor of the cap was rather below 0.5 normal. In the meristem the limit was slightly higher, but the regulation had not kept pace with that in the cortex; and in the latter it was not what might have been expected from the observations of Stange [a] on the roots of various European plants.

My experiments on the absorption of potassium nitrate conspicuously show that the absorbing activity of the coconut roots is little interfered with by a moderate concentration of the surrounding solution (up to at least 0.2 normal). This obviously fits it for life in its typical habitat; for, while the water in the soil near the sea, and even in the beach itself, is not usually saline, because its mass movement is seaward, yet strand plants are subject to inundation during storms, which sometimes bring an amount of sea water about their roots which would be fatal if they were more sensitive.

THE COCONUT LEAF.

Gross morphology and growth.—Aside from the cotyledon, which is a very short sheath at one end with an enormous absorbing structure at the other, the first leaves of the coconut are mere sheaths, resembling the bases of later leaves, but entirely destitute of any lamina. These sheaths are usually 4 to 6 in number, each being longer and less scale like than its predecessor. In vigorous seedlings they sometimes appear at intervals of less than one week, but as a rule the succession is slower. Their most rapid measurable growth is immediately after they emerge from the nut. The transition from sheaths to leaves may be abrupt; or there may be one or two, the upper part of which, after splitting, bends outward, like the rachis of a leaf, but develops no blade.

The succeeding leaves, 2 to 6 in number, do not become pinnate, but develop a lamina, which splits down the median line, sometimes merely forming a notch, but usually extending more than half of the length of the

[a] Stange, B.; Beziehungen zwischen Substrat-concentration, Turgor, und Wachsthum bei einigen phanerogamen Pflanzen. *Bot. Zeit.* (1892), **50**, 253, etc.

blade. The most rapid growth of the later of these leaves occurs at a period which is a week or more after their emergence. They are plicate in vernation, but the folds are shallow and are almost or entirely smoothed out when the leaf is fully expanded. The result of this is an increase in the area of the leaf, without a corresponding growth of its margin; and this, in turn, causes it to become convex on the upper surface and to curve outward, whereby its exposure to light is materially increased, and the stomata-bearing nether surface is protected against wetting by rain. The first ones of these split leaves are apparently sessile, with blades about 20 centimeters long; the later ones are short stalked, and the length of the blades may exceed 70 centimeters.

The transition from split to pinnate leaves is a gradual one. At first only a few of the lowest folds separate, the appearance of the greater part of the lamina remaining like that of one of the merely split leaves immediately below it; in succeeding ones the pinnate lower part increases at the expense of the compact upper part until the latter ultimately disappears. The number of leaves sharing in this transition varies considerably, 6 being a common one. In length they may be from less than 1 meter to a size considerably larger. The earlier leaves are all short lived, and, as each succeeding one is larger than the preceding, their dimensions on a young tree are constantly increasing. In cultivation the nuts are germinated collectively and the seedlings set out in their permanent places during the split-leaf stage. The increase in diameter of the mass of the bases of the petioles is constant, and as the leaves have sheathing bases, as the tree grows, the latter rise into the air as a false stem, resembling that of the banana or abacá; this false stem reaches a height of about 150 centimeters before the real stem or trunk is visible. For several years after the appearance of the trunk, the leaves continue slowly to increase in number and in length. When the first nuts appear, at an age of from five to nine years, the tree is bearing at least twenty leaves. Even after this time there is usually some increase in their size; in vigorous old trees the number is 25 to 30 or even 35; each of these leaves is from 5 to 8 meters in length, with about 80 pairs of pinnæ, large and small.

The following table shows the rate of growth of the scales and split leaves of a number of seedlings. The measurements are from a mark on the lowest visible sheath, the husk not being dissected away; therefore there may have been some of the oldest sheaths invisible and not represented; and the growth being basal, the mark on the lowest visible sheath can record no growth. The entire elongating region is always within the protecting sheaths of the lower leaves, so that zones marked on any visible part of the leaf retain their exact intervals. Leaf No. I is the one which was marked, the others being successively younger. The numbers in parentheses represent total length; the others, the growth during the time

elapsing since the preceding measurement. Blanks indicate no growth. All measurements are in millimeters. "G" is the girth:

Leaf No.		Dec. 15.	Dec. 22.	Dec. 29.	Jan. 5.	Jan. 12.	Jan. 19.	Jan. 26.
I	1	(9.5)						
	2		(11)	10	5	3		
	3			(22)	20	17	10	
	4					(65)	28	
II	1	(11)						
	2			(7)	2.5	1	1	
	3		(12)	14	11	11	9	8
	4						(18)	8
III	1	(11)						
	2	(37)	10	4	2	2	2	1
	3		(66)	23	14	11	7	4
	4		(67)	48	44	46	46	51
	5							(180)
IV	1	(20)						
	2	(40)	3	2	1			
V	1	(29)						
	2	(56)	7	2	2		1	2
	3	(103)	38	23	20	13	8	3
	4	(67)	39	36	41	53	38	29
	5				(89)	52	43	54
	G	(28)	3	2	4	1	3	4
VI	1	(16)						
	2	(46)						
	3	(80)						
	4	(109)	16	18	14	5	2	2
	5	(120)	51	53	53	48	51	33
	6				(142)	49	56	65
	G	(28)	6	1	5	2.5	4.5	3
VII	1	(5)						
	2	(46)						
	3	(103.5)						
	4	(204)						
	5	(469)	7	1	1	3	3	1
	6	(586)	109	28	33	4	1	8
	7	(399)	71	81	73	85	91	91
	8						(352)	92
	G	(60)	5	2	1	2	5	4
VIII	1	(38)						
	2	(126)						
	3	(390)						
	4	(633)	25	6	11	3		
	5	(342)	69	64	21	18	46	36
	G	(57.5)	2.5	3			1	

Under fair conditions each leaf of a young tree grows decidedly more rapidly than the next older one, and in seedlings which are of the size of the ones mentioned above, several leaves grow rapidly at the same time. While the plants represented in this table were under observation the growth of their roots was prevented by frequent moving. This injury was reflected by a slower development of the shoot before the measurements ceased. Each leaf had less than the normal advantage over its

predecessor, and its period of rapid growth was abnormally short, so that in most cases only a single leaf was growing vigorously on each seedling.

Working as I did in the open and therefore largely depending on nature for modifications of the environment, it was very difficult to secure any reliable data on the influence of the individual factors of the environment on so slow a process as growth. Of the plants represented in the foregoing table, those with even numbers were watered twice daily during the first two weeks. As compared with the alternate ones, which were placed in an otherwise drier place and which received as much as 1 millimeter of rain but once in fourteen days, the growth of the watered plants was much slower, but the relative rapidity of development was not affected by reversing the positions during the succeeding fortnight; from which it appears that the difference was inherent in the individuality of the plants, and that it is a matter of practical indifference to seedlings of the ages of the ones which I was observing whether they be given much water or very little. Observation of a seed bed where more than 5,000 nuts were placed to germinate justifies this conclusion. Differences in the exposure of different parts of this bed were not reflected in the growth of the seedlings. Until the area of the leaves permits an appreciable transpiration, the nut must contain all the water the seedling normally demands for its growth. If the husk is entirely dry the roots do not emerge from it, but this may as well be due to the extreme toughness of the dry husk as to the abnormal loss of water from the roots and to any inability on their part to absorb water. After this time a removal of the roots or a prevention of their growth by frequent moving stunts the development of the seedlings, and no amount of water will altogether obviate this result, though, of course, the injury is fatal only when excessive dryness or some other cause prevents the development of new roots. Whether the injury to the growth of the shoot of well-watered plants is correlative [7] or because enough water can not be absorbed is uncertain, but in either case the leaving of the seedlings in the germinating bed after the nut's supply of water ceases to satisfy all demands, will result in injury when they are transplanted, even under the most favorable conditions.

The available moisture determines the rate of growth of the leaves of older plants to the practical exclusion of the influence of all other factors. My work on these older plants began after the influence of the dry season was seriously felt. Drought interferes first with the growth of the youngest individuals, the larger one suffering less, in proportion to the depth and extent of their root systems. The following table shows the growth of one plant (A) the development of which had practically been arrested, and of another (C) which up to the time of observation had comparatively been but little affected. In each case a leaf tip barely protruded

[7] Kny: Correlation in the Growth of Roots and Shoots. *Ann. of Bot.* (1894), 8, 265. Townsend: The Correlation of Growth Under the Influence of Injuries, *Ibid.* (1897), 11, 509.

from the mass of bases. A stout stake was driven into the ground until its top was exactly even with the tip of this leaf. All measurements given are from the top of this stake and are expressed in millimeters. Increments since the preceding measurements are indicated by boldface type. The heights of the stakes were, respectively, 500 and 1,320 millimeters. The experiments began on February 8:

Date.	Leaf A.		Leaf C.	
Feb. 10	1		40	
Feb. 17	2.5	**1.5**	190	**150**
Feb. 22[a]	3	**.5**	306	**116**
Feb. 24	(16)	(13)		
Mar. 1	88	**85**	404	**98**
Mar. 10	311	**223**	550	**146**
Mar. 17[b]	494	**183**	665	**115**
Mar. 23[c]	659	**165**	772	**107**
Mar. 30	784	**125**	885	**113**
Apr. 6[d]	895	**111**	1,004	**119**

[a] Plant watered.
[b] Leaf begins to expand.
[c] Watered last, March 20.
[d] Marked part of both leaves expanded.

The following contains a more detailed tabulation of the growth of these two leaves for a portion of the time included in the preceding one and shows the relative growth by day and by night. All measurements given are the increments during the preceding periods:

Date.	Hour.	Leaf A.		Leaf C.	
		Day.	Night.	Day.	Night.
Feb. 22	6 a. m.				
	9 a. m.[a]			1	
	9 p. m.			0	
Feb. 23	6 a. m.				5
	12 m.			0	
	6 p. m.			1.5	
Feb. 24	6 a. m.				11.5
	6 p. m.			1.5	
Feb. 25	6 a. m.				15.5
Mar. 10	6 a. m.				
	6 p. m.	3		0	
Mar. 11	6 a. m.		19		15
	6 p. m.	3		0	
Mar. 12	6 a. m.		21		18
	9 p. m.	8		3	
Mar. 13	6 a. m.		15		13

[a] This interval follows the first watering of leaf A too promptly for the growth to be at all normal.

A few measurements at other times agreed entirely with those given above in demonstrating that the measurable growth very largely took place at night, the diurnal growth of the plants which were seriously

suffering from drought falling to nil. Indeed, a slight but unmistakable shortening occurred on certain of the days of observation. The reason for this strikingly unequal distribution of the growth is that the active transpiration during the day creates an internal scarcity of water and reduces the content of that liquid in the plant to such an extent that any considerable enlargement is impossible. A similar, but much less pronounced, daily periodicality of growth is reported for the bamboo,[9, 10] correlated with the relative humidity. Every factor which contributes to the more active transpiration during the day is also in part responsible for the cessation of growth.[10]

It is a very common practice in Mindanao to plant coconuts and abacá together, in the expectation that the abacá will support the commercial undertaking until the coconuts mature. This may be expedient, from a business standpoint, where the cost of clearing is the chief item in the establishment of a plantation; and after the first two or three years the coconuts suffer less than the abacá in this competition; but the maturing of the former is delayed by probably two years, and the trees are never as robust as those which were better illuminated from the start. The ultimate diameter of the trunk of a palm is determined in its youth.[12]

The heliotropism of the coconut is illustrated by the well-known disposition which trees along the beach have to bend toward the water (fantastically ascribed to the tree's love of the sea) and by the tendency of those around the outer edges of a grove to lean outward in every direction. This heliotropism is the more interesting because the actual growing region, where the curving takes place, is deeply seated below the visible tip and covered by the bases of many leaves.

The negative geotropism of the trunk causes a prostrate tree to turn upward with a curve the radius of which often does not exceed twice the ultimate diameter of the trunk. This abrupt curvature is rendered possible only by the harmonious reaction of many growing leaf bases, those beneath developing more and those above less rapidly than the ones in the middle. Each leaf base executes its own appropriate curve. These

[9] Lock: *Annals Bot. Gard. Peradeniya* (1904), **2**, 211. Not seen.

[10] Kraus (Das Längenwachsthum der Bambusrohre, *Ann. Jard. Bot. Buitenz.*, 1895, **12**, 196), working at Buitenzorg, with almost daily rain, found the diurnal retardation of the growth of bamboo slight compared with that reported here for *Cocos*.

[11] At least the larger proportion of the experiments which are supposed to show that light exerts a direct retarding influence on the growth of stems and leaves are questionable because they do not exclude the possibility of the direct influence of the illumination on the transpiration and a consequent indirect retardation of growth. While the immediate effect of light is to retard growth, adequate illumination is of course eventually indispensable for the healty development of the plant.

[12] The nuts in a seed bed are usually placed horizontally because the trunks grown from such seeds are supposed to be stouter. Drude, in *Natürlichen Pflanzenfamilien*, II, **3**, 3, states that some palms, such as *Sabal* and *Ceroxylon*, normally develop stouter trunks if their earliest growth is horizontal.

reacting bases are organically connected only by means of tissue which must completely have ceased to grow (it is not available for measurement), and the harmony of the entire reaction is no evidence of any communication between the units concerned in it.[13]

Anatomy of the leaf.—In describing the anatomy of the coconut leaf nothing need be said about the fibro-vascular tissue except that the finest longitudinal veinlets are hardly more than 0.1 millimeter apart, so that water in order to reach any cell of the parenchyma only needs to pass an exceedingly short distance by osmosis. The structure of the individual veins and veinlets offers no peculiarities.

The most striking structure in the leaf is what may be called the "hinge." Running ventrally for its entire length along each side of the midrib of the pinna is a narrow strip, sharply differentiated from any neighboring living tissue by its colorless contents. A crease along the middle of each of these strips makes the leaf thinner at this point than anywhere else, the colorless hinge tissue occupying more than half the thickness of the leaf but not entirely crowding out the green mesophyll. The epidermis of the hinge, as seen in transverse section, is remarkable for its exceedingly convex outer walls. The two accompanying figures (15 and 16) make this structure clear.

Because of the convexity of the outer walls of the individual cells, the wall of the epidermis, in this situation, as a whole is very much wrinkled; so that a bending or even a stretching can obviously be accomplished by a very slight and easy bending of walls at right angles, without giving rise to the uncompensated stretching of any one unit. Other parts of the leaf have the thick outer walls practically plane, and as any bending would involve the extension or direct compression of the whole of one of them these parts are practically rigid. Therefore, the crease mentioned above facilitates movement not only because it makes the leaf thinner at this point but also because it increases the convolution of the walls and reduces their resistance.

The active tissue concerned in the movements of the hinge is the colorless mesophyll. Its cells are large, and they have thin walls which are easily bent or even stretched. It is without intercellular space, so that the slightest alteration in the volume of the individual cells changes that of the entire tissue. The volume of the cells must obviously vary with their water content. When the leaf is well supplied with water the cells of the hinge are distended to their full capacity and it is open, thus holding the two sides of the pinna as far apart as possible. When the supply of water is insufficient the reverse takes place. By this means the exposure of the pinna to the rays from the sun or sky is lessened and a "dead air" space, though usually a very imperfect one, is formed under it. In both of these ways the further loss of water is checked.

When the pinna is losing water faster than it is being furnished from

[13] Cf. my paper, "The Geotropism of Split Stems," *Bot. Gaz.* (1900), **29**, 189.

below, the hinge responds before the cells of the green mesophyll begin to suffer. The explanation is as follows: All the cells hold their water through the osmotic activity of their contents; the turgor of the chlorophyll-bearing cells is such that plasmolysis begins in about 0.5 normal potassium nitrate, while the cells of the hinge begin to plasmolyse in less than 0.3 normal, as a consequence the latter will lose the greater amount of water in the shorter time, thus causing the hinge to close.

The actual behavior of the hinge is sufficiently illustrated by the following table, which gives the distance, in millimeters, between the edges of the two pinnæ, each measured 20 centimeters below the tip, at intervals, for two days:

Leaf.	Dec. 7.						
	7 a. m.	8 a. m.	9 a. m.	10 a. m.	11 a. m.	12 m.	1 p. m.
A	20.3	20.3	17.5	15	13	12.5	12.5
B	25	25	21	20	18	17	16.5

Leaf.	Dec. 7.			Dec. 8.		
	2 p. m.	3.30 p. m.	5.10 p. m.	6.30 a. m.	7.30 a. m.	8.30 a. m.
A	13	13	17	20.3	20.3	20
B	16	17.5	21.5	25	25	23

Leaf.	Dec. 8.							Dec. 9,
	9.30 a. m.	10.30 a. m.	12 m.	1 p. m.	2 p. m.	4 p. m.	5 p. m.	6.30 a. m.
A	16.5	13.5	12	13	12.5	13.5	16.5	20.5
B	21	18.5	16	16.5	15	17	21	25

As the accompanying records show,[14] the months between November and April were exceedingly dry at San Ramon. The influence of this climatic condition on the behavior of the hinge is shown by the following measurements, in millimeters, made on the same leaves at the same points one month later than the date of the preceding table:

Leaf.	Jan. 11.		Jan. 12, 7 a. m.	Feb. 8, 7 a. m.
	6.30 a. m.	7.30 a. m.		
A	18	18	18	17
B	23.5	23.5	23	21

The surface of leaf B was moist on the morning of January 11, and this fact demonstrated that its failure to open as widely as it did a month

[14] See hygrometric readings appended.

before had become chronic. This was due to the prolonged drought and not to the age of the leaf, for in seasons of fair precipitation the pinnæ lose none of their original power of expansion, at least until they are much older than the ones measured in these experiments.

Aside from the hinge, the mesophyll is differentiated into the green assimilating, and what, in deference to custom, I will call a water-storing tissue. The latter is composed of two layers of cells, immediately beneath the upper epidermis. The upper of these layers has the cells elongated at right angles to the length of the pinnæ, as is shown by figs. 17 and 18, while those of the inner layers are considerably deeper and larger. Both are almost perfectly transparent and their contents consists almost entirely of water. They form no inconsiderable part of the volume of the leaf. Their walls are not sufficiently thin either to be collapsible or to stretch very easily, so that only an insignificant part of the water which they contain can ever be available to replace any loss on the part of other cells. They are primarily rather to be regarded as a screen, the function of which is to mitigate the injurious effects of too extreme insolation on the underlying green cells. The same is true of the thick-walled, so-called water-storing tissue of many other plants.

The green mesophyll is but feebly differentiated. There usually are two layers which may properly be termed palisade cells, and about four more, the cells of which are irregularly placed; but the leaf throughout is too compact for any tissue appropriately to be designated as spongy. The turgor of the assimilating tissue is equal to about 0.5 normal potassium nitrate.

The cells of the epidermal tissue are approximately isodiametric in surface view, their least diameter being the depth. They are devoid of chlorophyll, and hyaline. Their turgor is about that of the hinge cells, but their heavier walls prevent any considerable variation in their water content. The outer wall of the upper epidermis is 6 to 7 μ thick; that of the nether, 5 μ. The surfaces are glabrescent.

The stomata of the coconut leaf are confined entirely to the nether surface, where they number about 145 per square millimeter. They average in size about 30 by 33 μ with an area for the pores and for both guard cells of 740 μ square.

As the accompanying drawing of the transverse section (fig. 19) shows, the stomatal apparatus is practically superficial, the back of the guard cell being sunk just enough to make room for a hinge in the wall outside it and to permit it to move without interference from the thick, outer wall of the epidermis. The mechanism is exactly that of Schwendener's type of *Amaryllis*.[15] [16] The back wall of the guard cell is so thin that it collapses and wrinkles in plasmolysis. The ventral half of each, namely, the one next to the pore, is strengthened by the powerful ridges of

[15] Schwendener: Ueber Bau und Mechanik der Spaltöffnungen, *Monatsber. Akad. d. Wiss.*, Berlin, 1881, 833.

[16] Copeland: The Mechanism of Stomata, *Ann. of Bot.* (1902), **16**, 339.

entrance and exit and by the neighboring, comparatively heavy parts of the wall. Anticlinal walls never strike the guard cells midway. A more detailed explanation of the mechanism of these stomata would be superfluous here. However, there are some interesting features about their turgor and their behavior under changes of illumination and with the gradual withdrawal of their water which render it worth while to introduce some measurements. The agent employed by me to withdraw the water was potassium nitrate. The solutions were 0.3 normal, 0.5 normal, and normal. Measurements are in microns:

	In pure water.	0.3 normal.	0.5 normal.	1 normal.
Stoma:				
Length	34	35	35	34
Width	31	30	28	26
Pore, width	5	3	1.5	0
Ridge of exit	9	7.5	7	6.5

The normal solution plasmolysed the guard cells and caused the contents of other epidermal cells to collapse until they occupied hardly half the previously visible area. Remaining ten minutes in this solution killed many of the former, and others opened the pore only after the solution was replaced by water and the slide exposed to the direct sunlight. The two stomata described below half opened in water and completely in the sunlight. Measurements are in microns:

Leaf.		Direct sun.	Obscure light.		Microscope stage.	
			15 minutes.	75 minutes.	10 minutes.	15 minutes.
A	*Width:*					
	Stoma	32	31	29	30.5	31
	Pore	5	4	2	3	4
B	*Width:*					
	Stoma	30.5	30	30	30.5	31
	Pore	5	3	0.5	2	3

It appears from these measurements that seventy-five minutes in quite diffuse light affects the degree of opening of the pore about as much as does immersion in 0.5 normal potassium nitrate solution. The recovery of turgescence with better illumination occurs with amazing promptness when one considers the great change in turgor which precedes and causes it. In this experiment, as is always necessary when stomata are under prolonged microscopic study undertaken with sufficient care to permit of accurate measurements, they are immersed in water. When they are in the natural condition on the living plant they respond much more quickly and thoroughly to the withdrawal of the light, as is shown by experiments to be reported below, in which the rapidity of transpiration is determined by the cobalt test.

While all recent writers on the subject have assumed that changes in turgor are responsible for the changes in turgescence with variations in the illumination, it has never, so far as I am aware, been demonstrated that the turgor really does vary.[17] I myself have tried to measure such a change with divers plants, but without success. However, with the coconut it is easy to determine that the turgor is much higher in light than in darkness, though the actual differences are rather inconstant. The turgor of a single pair of guard cells can be demonstrated to change during a prolonged experiment; but as this involves plasmolysing the pair at least twice, and, as a rule, subjecting it to several strong plasmolysing solutions, each of which must be given time to act, the cells are likely to suffer changes from this treatment. The evidence taken from the observation of many different stomata, in their natural condition, at different times of day, is more valuable.

The turgor on sunny afternoons is usually about equal to normal potassium nitrate. Sometimes it exceeds even this high figure. Thus at 3.30 p. m. November 25 these measurements, in microns, were made:

	0.5 normal.	0.7 normal.	1 normal.
Pore, width	2.5	1.5	Closed.

While the particular stoma under observation was not measurably open, about one in five on the section was open to the extent of at least 1 μ, the plasmolysis of any guard cells being very doubtful; but when the normal potassium nitrate was replaced by glycerin, plasmolysis was evident everywhere, and all stomata were closed. If they were examined early in the morning the guard cells were usually found to have their turgor equal to somewhat less than 0.7 normal potassium nitrate but rarely below 0.6 normal. In direct sunlight the increase is an immediate one.

The action of prolonged darkness is very different from that of the mere nocturnal lack of light. A leaflet was kept in darkness, inside a wooden cylinder, for ten weeks, at the end of which time its turgor, as compared with that of a neighboring pinna under ordinary conditions, was:

Name.	From darkness.	From normal leaflet (in morning).
Epidermis		
Guard cells		
Parenchyma		

[17] The term "turgor" is used to express the osmotic pressures of the *internal* fluid of a cell. On the other hand, the expression "turgescence" applies to the strain resulting from the interaction of the force of the osmotic pressure (the diffusion tension of the solute) on the one hand and that of the resilience of the cellulose membrane on the other. Copeland, *Ann. of Bot.* (1902), **16**, 330.

In my paper, "Ueber den Einfluss von Licht und Temperatur auf den Turgor,"[18] I showed that in leaves growing or grown in darkness the turgor is higher than in normal ones; but the pinna of the coconut which I had under observation did not, at least in area, grow during the experiment, therefore the explanation in this case must be a different one.

TRANSPIRATION.

Three general methods have been used in research on the transpiration of plants: First, measuring the water absorbed by the subject of the experiment; second, determining the loss in weight of the subject and its container; third, ascertaining the amount of transpired water after it leaves the plant. I have used all of these in my work on the coconut.

The first method is of the least value because it does not directly measure the transpiration, and because the amount absorbed and that given off in a given time are not necessarily equal. I employed it in some preliminary experiments only, when my equipment did not permit the use of either of the others. As the pinnæ which served as subjects always lost weight almost from the beginning of the experiments, absorption being less rapid than transpiration, the method is inapplicable when any measure of accuracy is desired. It will, of course, be understood that all ordinary means of keeping the absorption normal were employed.

In all experiments on the transpiration of this plant in which the subject is to be weighed the use of single pinnæ is practically compulsory, for even young seedlings are so heavy that the loss of water from the limited leaf area in such time intervals as one hour would escape notice. Entire leaves have the same disadvantages as do pinnæ, and besides they are most unwieldy. When it can be used at all, the determination of the loss of weight of subject and container is the most reliable method of ascertaining the transpiratory activity of any plant, and when, as in this case, the use of whole plants is impracticable, it is usually feasible, with proper care and precaution, to be sure that isolated parts of them behave, at least for some time, as they would in their natural positions. I have used this method in the larger part of my work, but, in contrast to experience with other plants, have found it quite impossible to make single, isolated pinnæ of the *Cocos* maintain the normal rate of transpiration for more than a very short time. Leaves cut under water, with the cut surface at all times protected from exposure to the air, approximated normal transpiration but little if at all more closely than those treated without this care. This has repeatedly been my experience. One rather extreme illustration will suffice to demonstrate it: The cut surface was never exposed to the air. The first weighing was immediately after cut-

[18] *Dissertation*, Halle, a. S., 1896.

ting. The leaf was exposed to direct sunshine during the greater part of the time:

Interval.	Loss of weight.	Loss per minute.
Minutes.	*Gram.*	*Gram.*
6	0.15	0.025
13	.17	.013
50	.26	.005
310	.70	.002

The average loss per minute during the last interval was only 8 per cent of that during the first. Transpiration does not usually cease so promptly, and the relative loss is less, the longer the first period is made. It is a general rule, in experiments of this kind, to permit the subject to stand for a time after cutting, and thus to become accustomed to its new conditions before observations really begin. If this is done with *Cocos* the rapid initial transpiration can not be observed, and thus the abnormality of the results obtained must escape suspicion. Many of my tables, which seemed satisfactory when made, are valueless on this account. When the transpiration of a leaf varies during a single half day by 92 per cent of its maximum activity, independently of any change in the environment, it is obvious that any modification of the latter must have results which are comparatively too insignificant to be studied with any confidence. Therefore, I was forced to seek a means of preventing the usual reaction to the cutting of the pinnæ.

The water in which cut pinnæ stand ceases to be clear, becoming a pale, often opalescent, brown. This is sometimes evident within half a day after cutting, but usually it is not seen until a day or more has elapsed.[19]

Suspecting that an exudation from the cut surface (though none was visible) might be preventing the absorption of water, I tried renewing the cut. It was doubtful if the transpiration was accelerated; certainly such acceleration was not enough to be applicable in drawing any conclusions.

It was shown by Janse [20] that, while boiling a part of the path of the transpiration stream ultimately results in interference with the movement of water, this result is not immediate, and is due to changes in the part remaining alive, not in that killed. It occurred to me that boiling the bases of the pinnæ might prevent the checking of their absorption for at least a few days. As a matter of fact, the cessation was less immediate and less complete in pinnæ so treated than in others, but it was still so great that the results obtained by this method alone are far from satisfactory. They are shown in the next table.

[19] This is a conspicuous exception to Sachs's statement that nothing escapes from such cut surfaces into water.

[20] Janse, J. M.: Die Mitwirkung der Markstrahlen bei der Wasserbewegung im Holz, *Jahrb. wiss. Bot.* (1887), **8**, 1.

The fact that neither renewing the cut nor killing the lower ends of the pinnæ prevented the practical cessation of the transpiration would suggest that this cessation is due to some reaction on the part of the stomata, but this can hardly be true, for, as already noted, the cut pinnæ lose weight during the experiments. The only explanation I can suggest for the persistent refusal of the cut pinnæ to absorb water at least as readily as they normally secure it from the rachis of the leaf is that a pressure of less than one atmosphere within the tracheæ is a condition for the ready movement of water through them, and that offering water to the pinnæ at a higher pressure than the usual one, instead of making them absorb more, is in itself the cause of their absorbing less. I am not ready to support this suggestion here, and know that it is contrary to the generally accepted opinion that water travels through wood with equal readiness regardless of whether the motive force is applied as a pressure (above one atmosphere) or as a suction (less than one atmosphere). It seems to me that this may be true in some cases and not in others, depending, for one thing, on the amount of air in the conducting elements.

Determining the water given off by leaves by absorbing and weighing it is a method which has long been in use. A decade ago, in a paper not accessible to me at the time I carried out this work, Stahl introduced the use of anhydrous chloride of cobalt, the rapidity of transpiration being estimated by its change in color from blue to pink as the salt absorbs water, because cobalt salts are blue when anhydrous, red when hydrated. As standards I used pieces of absorbent paper saturated with cobalt-chloride solution, one set not quite as blue as it would be if entirely anhydrous, the other not as red as if entirely hydrated; these sets were separately sealed in glass vials. While changing from the color of one of these to that of the other, a piece having an area of 100 square centimeters absorbed 0.46 gram of water. By the use of this cobalt paper the transpiration of pinnæ in their natural positions on the tree could be tested, the evil effects of cutting being entirely obviated. However, the method has compensating disadvantages.

The cobalt paper must be directly applied to the transpiring surface, and it must be protected against the possibility of absorbing water from the atmosphere. This is accomplished by holding it in place with glass (microscope slides serve the purpose well), the latter in turn being held by clamps. This method is likely to make the transpiration abnormal by interfering with the wind, by cutting off some of the illumination, and by placing a portion of the leaf, at least for a part of the time, in an abnormally dry atmosphere.

Transpiration is exceedingly sensitive to changes in the illumination, so much so that if a slide which is locally corroded be used over either surface, the paper under the etched spot will be noticeably slower to turn red; therefore clear and perfectly clean glass must be used. However,

there is no avoiding interference on the part of the cobalt paper itself; but nearly all of the light to which the plant has access comes from above, and the disturbing effect of cutting off that from below is correspondingly moderate.

The wind affects the transpiration in two ways—by constantly changing the air immediately outside the stomata and by agitating the leaves and thus causing a circulation within the intercellular spaces and an egress and ingress through the stomata. The disturbance of the first of these effects is inoperative in this case because of the drying action of the cobalt paper; and as the pinna as a whole remains fairly movable, only the small part between the slides being rigid, and the intercellular spaces are continuous, the interference with the circulation is at most but partial. The disturbance of the transpiration by cutting off the wind is therefore not a serious matter.

That the cobalt-chloride test of transpiration places the leaf in an abnormally dry atmosphere is a great and unavoidable objection. Even if the plant did not react to this condition other than as a surface of water would, namely, by more rapid evaporation, this error would be very difficult to control; for while the blue paper must constantly surround itself with very dry air, this medium becomes damper as the paper turns red.[21] In practice, the matter is far from being as simple as if we were studying evaporation from a water surface only. When the cobalt paper is applied to a surface with open stomata it suddenly makes an increased demand on the water vapor in the intercellular spaces which are in immediate contact with the open pores, and most particularly on the water in the guard cells themselves. An abnormally active escape results, this in turn causes the stomata to close, checking the loss, and this process presently brings the transpiration below the normal. Thus, the decreased illumination and abnormal dryness work together in reducing the transpiration, and their combined effect is to cause the paper to change color, at first more rapidly than the normal transpiration would make it do so, but afterwards much more slowly.

That the rapidity of reddening of the cobalt paper comes far from indicating the actual rapidity with which the plant loses water is clearly shown in the last preceding table, in which the time intervals are those required for the reddening to take place. As the first column shows, the initial reddening took place in one-fiftieth of the time consumed in the last interval. The second column shows the relation of the reddening to the actual, though abnormal, transpiration. The area of this pinna was 75.4 square centimeters. Of this, 19 square centimeters was under the slide, leaving 56.4 square centimeters free; 56.4 centimeters of cobalt paper would absorb 0.26 gram of water in changing color. Evidently

[21] Very soon after paper in contact with actively transpiring leaves is really red, water begins to precipitate on the glass.

the cobalt paper withdrew water from the leaf during the first two periods more rapidly than it transpired from the free surface but much less rapidly during the last period. The change from an acceleration to a retardation occurred in about half an hour; it often appears more quickly. The measurement of transpiration by weighing cut pinnæ and their container was deemed unavailable when this experiment showed that the rate fell during a few hours to 8 per cent of the initial. As determined by the cobalt test, the rate fell during the same experiment to less than 2 per cent. This exhausts the really distinct methods of making continuous direct determinations of the transpiration of a single subject.

Neither of these usually reliable methods being alone available in working on the coconut, I next had recourse to combinations, attempting to check a continuous experiment with one method by applying frequent corrections obtained from observations by the other; thus at the same time having the advantage of accuracy in the weighing method, and that of working with uninjured pinnæ on the tree by the cobalt test. I first tried to reach these ends by determining at intervals of several days the loss of weight of pinnæ placed in bottles of water, and at the same time comparing the rate at which cobalt paper was turned red by these cut pinnæ with that at which it was altered when it was applied to pinnæ *in situ* on the tree. My most satisfactory experiments of this kind furnished material for the following table:

The pinnæ A, B, and C were cut on the afternoon of January 17 and the cut ends killed by insertion in nearly boiling water. The leaf D was freshly cut at 2.30 p. m. January 18, and its ends not killed. All weights are in grams. The loss was determined regularly at one-hour intervals during the day. The bottles were hung in the tree, putting the pinnæ as nearly as was possible in natural conditions. The bottles themselves were shaded to prevent heating.

Date.	Hour.	A.	B.	C.	D.	Behavior of cobalt paper.[a]
Jan. 17	5.30 p. m					
Jan. 18	6.30 a. m.	....	0.12	....	....	
	7.30 a. m.	0.03	.02	0.02	....	Red in 8 minutes; leaves were damp.
	8.30 a. m.	.14	.09	.09	....	C and T change equally; not completed in 60 minutes.
	[b]9.30 a. m.	.10	.08	.09	....	A and T the same.
	10.30 a. m.	.23	.13	.09	....	B red in 40 minutes; T in 26 minutes.
	11.30 a. m.	.72	.42	.27	....	One third faster on T than on A.
	12.30 p. m.	.61	.40	.40		
	1.30 p. m.	.71	.63	.50	....	A red in 55 minutes; T in 13 minutes.
	2.30 p. m.	.43	.74	.51	....	A red in 55 minutes; T in 20 minutes.
	3.30 p. m.	.53	.50	.35	0.51	D red in 45 minutes; T in 13 minutes.
	4.30 p. m.	.39	.86	1.02	1.17	D red in 17 minutes; T in 13 minutes.
	5.30 p. m.	.14	.16	.12	.41	D red in 40 minutes; T in 18 minutes.
Jan. 19	6.30 a. m.	.10	.05	.09	.10	(These are totals for 13 hours darkness).

[a] T = Tree. [b] Sun strikes A at 10.20, B at 10.25, C about 10.40.

During the following day the transpiration was much slower and too abnormal to be worth reporting in detail. The totals for the twenty-four hours ending January 19 and January 20 were:

Ending—	A.	B.	C.	D.
Jan. 19	4.13	4.10	3.55	----
Jan. 20	1.58	1.71	1.33	2.02

If now the transpiration of a pinna *in situ* be computed from the loss of weight by pinnæ in water, and the relative rapidity with which these and the former turn cobalt paper red, the following result is obtained:

Hour.	Grams.	Remarks.
7.30 a.m	0.03	Observed for A.
8.30 a.m	.14	Do.
9.30 a.m	.10	Do.
10.30 a.m	.23	Do.
11.30 a.m	.96	4/3 by 0.72.
12.30 p.m	.81	4/3 by 0.61.
1.30 p.m	3.02	4.25 by 0.71.
2.30 p.m	1.19	2.75 by 0.43.
3.30 p.m	1.78	3.5 by 0.51.
4.30 p.m	1.53	17/13 by 1.17
5.30 p.m	.91	2 2/9 by 0.41
Night	.10	Observed for A.
Total	10.30	For one pinna and one day.[a]

[a] The free area of leaves furnishing this figure averaged about 120 square centimeters, the rate therefore equaling 8.57 grams for 1 square decimeter. Haberlandt (Anatomisch-physiologische Untersuchungen über das tropische Laubblatt, *Sitzber. Wiener Akad.* (1892) **101**, I; 804, 807) found a rate for *Cocos* at Buitenzorg of 0.89 gram per diem per square decimeter of surface.

Allowing 150 pinnæ to the leaf and 25 leaves to the tree, this indicates a total daily transpiration for the tree of 38,551 grams. My estimates made in this way have ranged between 28 and 45 liters. These calculations are based on determinations made on sunny days, and some of them are doubtless higher than the average transpiration of the tree for all days. On the other hand, it is to be observed that no allowance is made for the fact that not all parts of the pinnæ under experiment were free to transpire.

Another way of combining the cobalt test with the weighing method is to use fresh leaves at frequent intervals. This combination offers the advantage that the transpiration of the subjects weighed is never very much below the normal, but the disadvantage that it is difficult, with such a frequent change of subjects, to apply a control based on the continuous use of the same pinna. In practice, if the cobalt paper is always applied to a fresh part of the pinna, it will turn red once or twice after the pinna is cut, at practically the same rate as before. Under ideal conditions this method will furnish really accurate results, but the test of a method is

its usefulness under unfavorable circumstances, and under them the lack of continuity becomes too great an objection; in addition, the observations demanded at short intervals on several pinnæ at once require a dangerous haste in manipulation. This method is serviceable where immediate results will answer, as, for example, in testing the effect of shading. Estimates of the total daily transpiration by this method, based on determinations made in the sun, run higher than those just given—sometimes as high as 75 liters per diem.

After this necessarily prolonged discussion of method, a brief consideration of the relative transpiration from the upper and nether surfaces of the leaves, the influence of their age on their transpiration, and the effect exerted from without by the illumination and the wind will be possible.

Almost the entire transpiration of the coconut is through the stomata of the nether surface of the leaf. In experimenting on the transpiration from the upper surface, and at no other time, have I found it necessary to seal the edges of the glass slides to prevent interference by the moisture of the atmosphere; of course, it was also necessary to guard against the passage of moisture from the nether surface to the upper. These precautions being taken, it requires at least six hours of continuous sunshine to enable the cobalt paper to change color. If the leaf is placed in the shade or in the dark, the hydration is somewhat slower. On January 21, a day when there were occasional clouds, the average time of reddening, when the paper was placed against the lower surface, was eleven minutes, but against the upper, seven hours; this interval, from 9 a. m. to 4 p. m., was required for the change; and paper blue at 11.25 was still of the same color at 5.30, but red at 8.

Experiments were made on the transpiration of leaves which were just full grown, those about six months older, and those a year beyond maturity. Two series of determinations were undertaken with the individuals of each age. These varied in detail, as is true with all of this work, but the relation was constant—the leaves six months beyond maturity transpired rather less than those which had just grown, while those a year old were decidedly the most active of all. For example, the total transpiration for seven hours, from 9.20 a. m. to 4.20 p. m., February 14, was—

	Grams.
Mature leaf	2.70
Six months older	1.68
One year older	3.37

The result for the leaf of mean age is too small; this is due to its being the first to become greatly abnormal. The totals for 4½ hours, from 1.50 to 5.20 p. m., February 15, were—

	Grams.
Mature leaf	0.78
Six months older	.75
One year older	1.50

When I first observed this phenomenon I was surprised that the oldest leaves showed the most active transpiration, but a few weeks later a paper by Bergen [22] was received showing that the coconut is not peculiar in this respect. The figures given above are not corrected for area; if this were done it would still further emphasize the difference, because in both cases the oldest leaves had the least exposed area. The relative activity of cut pinnæ and those *in situ* was at first exactly the same in all cases and so demanded no correction, but the oldest leaves were always the last to show an extreme depression. The transpiration from the upper surface was slightly more active in the latter, but it was not enough so to account for any great proportion of the extra quantity. A considerable part of the total area of the oldest leaves was occupied by small, scattered brown spots, and the leaf was dead two months after these observations. The tree was a young one.

Thirty or more determinations of the transpiration during the night have all shown concordant results, the rate being about 1 per cent or even less of the greatest during the day; the total transpiration for an entire night was about one-tenth of that during one hour of sunlight at midday. Three factors are responsible for this great nocturnal depression—the darkness, the lower temperature, and the higher relative humidity. The complete experimental analysis of these three factors was practically impossible, but the cobalt test, being independent of the moisture of the environment, is capable of showing the inflence of the illumination independently of the relative humidity.

By this test it has repeatedly been proven that a very slight shade will to a certain extent almost immediately depress the transpiration. Of course, actual darkness has a very much greater effect. In using the cobalt test I held the glass slides to the leaf with cork clamps, and therefore the spot immediately between these was in approximate darkness. When, in the first test, the paper reddened in about four minutes, the change was not appreciably hindered by the cork; but if this first test required more time, and always during subsequent tests, the darkened part of the paper was very evidently slower in turning. Beginning at 9.18 a. m. January 21 the intervals required for reddening were ten minutes and nine minutes; then, with a light haze over the sun, fifteen minutes; all these for the illuminated part of the paper. After the last determination, the paper was left until the part under the cork was reddened; in about forty minutes water was precipitating on the glass over the lighted leaf, but the darkened paper was still bluish, only becoming as red as the standard after ninety-five minutes—that is, it took more than six times as long to change as it did when a mere haze weakened the light.

[22] Bergen, J. Y.: Relative Transpiration of Old and New Leaves of the Myrtus Type. *Bot. Gaz.* (1904), **38**, 446. "The leaves of six out of the eight species studied transpire more for equal areas when fifteen to eighteen months old than they do when they have just reached their maximum area (i. e., at three or four months)."

In observing another leaf, the intervals at the same time were respectively fourteen and eighty-two minutes, the ratio being practically the same. The effect of the passing of a cloud before the sun was observed very many times; it naturally varied with the depth of the shading. In similar cases the test by weighing shows a depression in transpiration, but I could detect no additional one to be ascribed to the higher humidity.

It is clearly in large part because the direct sunshine heats the leaf above the temperature of the surrounding air that the transpiration is so much more rapid in it than in the brightest diffuse light. The following table shows the extent of this overheating. The temperature was determined by tying a leaf backward around the bulb of a thermometer:

Hour.	Temperature.		
	In shade.	In sun.	In leaf.
	°	°	°
7 a. m.	20.3	21.8	21.9
8 a. m.	24.3	25.2	27.4
9 a. m.	26	30.7	33.1
10 a. m.	26.9	32	35.4
11 a. m.	27.8	31.5	[a]34.7
12 m.	28.3	34.7	37.7
1 p. m.	28	30	[b]31.5
2 p. m.	28.5	31.5	38
3 p. m.	28.8	31	36.7
4 p. m.	28.6	30.6	36.4
5 p. m.	27.7	30	34
6 p. m.	26.6	27.6	28.5

[a] Light cloud. [b] Cloudy.

How great a difference in evaporation, as a merely physical process, these differences in temperature will exert is shown by a consideration of the variation in the tension[23] of water vapor with changes of temperature. Thus, at noon the temperature in the shade was 28.°3; at this point the tension of water vapor is 28.560 millimeters; at the temperature of the exposed leaf, 37.°7, the vapor tension is 48.463 millimeters; at 11.30 a. m., with a temperature 28.°4, the relative humidity was 66. The tension of vapor in the air at that time was 18.89 millimeters, making a relative humidity for the temperature of the leaf of only 39; the unsatisfied possible tension of vapor in the air was 9.69 millimeters in the shade, while it was 29.583 millimeters for the leaf.

The actually observed excess of transpiration in strong, direct light over that in the shade was greater, as a rule, than that of evaporation from a water surface under the same temperature conditions; the change from a light haze, under which the leaf is already somewhat overheated, to full illumination, frequently multiplying the rate of transpiration by four. This extra effect may in part be due to the action of the stomata, and must in part be ascribed to the expansion of the gas in the intercellular spaces, with the consequent ejection, as the leaf is warmed, of a portion

[23] *Tables* of Landolt and Bernthsen.

of this gas loaded with moisture. Of course, the opposite change in the volume of this included air would take place as the leaf cools.

It was impossible for me to make observations of any great value on the influence of the wind, because I could not regulate or measure its velocity. With a good subject, the concomitant use of the cobalt test and the weighing method should make it possible accurately to analyse the wind's influence, showing how much is due to mechanical agitation and how much to the constant change of the air outside. But no work on the coconut is sufficiently accurate and reliable for such an analysis. As was to be expected, the wind made a much greater difference in the transpiration of the leaves which were exposed to the greatest illumination than it did in that of the shaded ones. Thus, in one instance, the transpiration in direct sunshine was four times as great in a wind I estimated to be at 5 miles an hour as it was in a calm; but the increase was usually not more than 100 per cent. In the shade, a wind of this velocity added less than 50 per cent to the transpiration. I was unable to cut off the wind from a shaded plant without further interference with its illumination.

Any estimate of the total water transpired by entire trees can not be more than a rough approximation, because, aside from all possible inaccuracies in the observations on individual pinnæ, different days and seasons are unlike; and different neighboring trees, as well as different parts of the same individual, interfere with each other's transpiration. For these reasons any estimate based on observations made entirely in direct light must be too high. As already stated, some calculations obtained in this way are as high as 75 liters per diem. In the experiment from which the estimate of 28 liters was obtained the pinnæ were under as normal conditions as possible, taking their share of shading with the other pinnæ of the tree and being under check by observations on pinnæ in the natural position. The day was bright, but was not quite cloudless, and not especially warm.

At the rate of 28 liters per diem the annual transpiration is 10,220 liters. In this volume of water the plant takes up the mineral food to be used in its permanent growth and enough more to cover the annual loss in the nuts and cast leaves. The amount of mineral food permanently bound up in the growth of the stem and roots can not be very considerable, and that in the roots which die is already in a place to be absorbed again. The average dry weight of a fallen leaf may roughly be put at 3 kilograms, of which 8.5 per cent is ash and nitrogen. Allowing a fall of 16 leaves per annum, the loss of matter taken up in solution by the roots is 4,080 grams. In each nut the tree loses ash as follows:

	Grams.
In the husk	33.84
In the shell	3.36
In the copra	13.83
In the milk	5.97
Total	57.02

If the tree produces but 20 nuts per annum, which is more than the recent average at San Ramon, the loss of mineral matter in these is 1,140 grams and the total loss in leaves and nuts 5,220 grams. If this were absorbed in 10,220 liters of water the concentration would be 0.051 per cent. This is considerably above the average concentration to be found in ground water, as determined by analyses from water in wells and springs, but as a general proposition the water in intimate contact with the ground particles, and, when there is but little water in the soil, all of it, will be more concentrated than that which will run freely from wetter ground; and the valuable mineral food of plants is absorbed from such dilute solutions in greater proportion than is the water in which it is dissolved.

Effect of drought.—The season during which I carried on my work at San Ramon was characterized by extreme dryness, and this condition has interfered with my study of the plant's normal physiology, but at the same time it has given me an opportunity to observe the injury done by the abnormal conditions. The following table contains my measurements of the rainfall for this year and Mr. Havice's for the corresponding months a year ago:

Month.	1904-5.	1903 4.	Relative humidity, 1904 5.
	mm.	*mm.*	*Per cent.*
November	(15-30) 91.5	(1-30) 208	79.9
December	2.5	260	79.2
January	30	32	75.65
February	1	131	72.35
March	0	232	75.43
April	12.5	92	74.9

The third column gives the average relative humidity at or near the beach at 11.30 a. m. for November and at noon for the remaining months. Details as to the rainfall and humidity during my work are presented in the appendix to this paper. While the dryness of the air certainly has some direct effect on the coconut trees—for example, in influencing the movement of the hinge, without regard to how well the roots may have been supplied with water—I do not believe that serious damage is ever done to the tree except by the dryness of the ground. In other words, *trees judiciously irrigated have nothing to fear from a drought, however severe.*

The cultivated part of the San Ramon farm is well supplied with ground water, which, as a rule, finds the surface through a number of large springs. Two months after the drought began, some well-cultivated spots were still wet from below every morning. During November, December, and January, I frequently examined the young tips of roots, and through these months there was no important change in the condition of the ground and accordingly none in the roots. After the latter

part of December there was a rapid drying back of the streams running to the farm from the mountains, and the desiccation of the ground became rather abruptly evident a month later. Through January, surface cultivation kept all but the most porous ground in good condition, but after this time it was practically useless so far as the soil was concerned. By the middle of March the soil, where it was not sandy and ready to crumble, was as hard as if baked, and under the thoroughly cultivated surface it was full of fissures as much as a centimeter in breadth. The hardness was shown by the behavior of main roots nearly 1 centimeter in diameter, to whose disposition to grow in a straight line the tree owes its firmness. These, upon entering the cracks, turned almost at right angles and started to follow them.

In such a soil it is obvious that, in a short time, growth will be suspended. On March 21, I was unable to find any roots apparently normally active. The cessation of growth had been accomplished, or initiated, by the shortening of the growing region until the hardened hypodermis had advanced to within the root cap, obliterating the white absorbing surface. The disappearance of the absorbing region in the small branch roots, with short caps, was at first less complete, but by April 11 that portion remaining unlignified at the tip even of these was more or less flaccid, even in the early morning.

The turgor in the cortex of these roots equals nearly 0.4 normal potassium nitrate. Approaching the meristem it is higher, probably 0.5 normal. In the cap and epidermis I was unable to determine it. It will be noticed that the increase in turgor caused by desiccation and cessation of growth is more than half what it is when the cessation of growth and immersion in 0.5 normal potassium nitrate act together. This shows what proportion of the increase in the latter is directly due to the absorption of the salt.[24]

Of course, the roots of a tree do not all suffer alike, because different strata of the soil do not become equally dry. I tested the amount of moisture in the soil on April 11, at depths of 20, 60, and 100 centimeters, determining the weight lost by drying at a temperature of 40.3° C., the dew point being 25.5° C. The loss was—

At depth of—	Per cent.
20 centimeters	16.6
60 centimeters	21
100 centimeters	[25] 23.2

[24] For the influence of rather concentrated solutions on the turgor of immersed roots, see Stange, in *Bot. Zeit*, 1892. For the influence which the mechanical prevention of growth exerts upon the turgor, see especially Pfeffer, *Ueber Druck- und Arbeitsleistung*, 1893. For a general treatment of the dependence of the turgor upon the rate of growth, see my paper, *Ueber den Einfluss von Licht und Temperatur auf den Turgor, loc. cit.* 1896.

[25] The difference in *available* water is much greater than these figures would indicate, for at 20 centimeters in depth the soil is the hard clay already mentioned, while at 100 centimeters it is a sandy loam, crumbling readily; at 60 centimeters it is intermediate in character.

A very large part of the roots of the coconut grow in the stratum between 20 and 50 centimeters, and a tree to which water is available only at a greater depth must suffer. If conditions exist which permit roots to grow only at a greater depth, the obvious result will be a larger proportion in this deeper situation; in this way trees growing in dry places adapt themselves to their environment. However, *trees which are compelled to adapt themselves to unfavorable conditions of any description can not be expected to be prolific.* This is well illustrated by the dearth of nuts on the *Cocos*-clad hills about Romblon and Masbate. The water which can be drawn from a dry soil contains a greater proportion of mineral substances dissolved in it than that which is available when the soil is wet, so that the proportion between the quantity of available mineral food and the amount of water absorbed is not constant.

The shoot suffers from the inactivity of the roots. The influence of the drought on the growth of the leaves, and on the action of the hinge, has already been shown. The leaflets, which under these conditions are more folded, absorb less light, so that the leaf area which the tree has at its disposition is less efficient in photosynthesis. A normally active tree produces from twelve to twenty-four or more leaves a year. After December, during this drought, no new leaves appeared on trees which were less than 2 years old, and not more than one on any tree less than 5 years old. As a general rule, the older the tree the later it begins severely to suffer, the probable cause being that its roots run deeper than do those of the younger ones; but the growth of the leaves of individuals of all ages was very evidently retarded during February. This, in itself, would result in a decrease in the number of leaves borne at one time; but another factor is at least equally efficient in bringing about this result. The old leaves of the coconut are cast in a succession which, in adult trees, normally keeps pace with the appearance of the young ones, so that the number present at any one time does not materially vary. The internal factors causing the fall of the leaves have never been investigated, but there is no doubt that dryness is one of them. The "physiological dryness" caused by the outside drought naturally finds expression in a more rapid aging and falling of the leaves. In fact, the first, and for a long time the only, noticeable symptom of dryness is the number of leaves pendant or falling. It has already been noted that trees without a rather indefinite minimum number (say, twenty) of leaves, have not the vitality which is necessary to ripen nuts. An individual with only approximately this number will naturally not bear many. A retardation in the production of leaves and an acceleration in their loss, when acting together, will rapidly bring even the strongest trees toward this limit.

The flowering branches are formed in the axils of the leaves, and the formation of fewer of the latter must in itself ultimately result in the growth of fewer of the former. However, in practice, the development of these branches themselves is dependent, like any other growth, on a

sufficiency of water; it is arrested at the same time as is that of the younger leaves, long before the ones formed during the drought would bear flowers on their axillary branches, even in the most favorable weather. Thus the number of branches whose pistillate flowers were "opened" on a certain tree during the six months ending with April, 1905, was six, whereas during the preceding six months it had been nine. The flowers do not open until more than six months after the first appearance of the subtending leaf.

The number of nuts which can be borne depends upon the number of fruiting branches, and on every branch there are more pistillate flowers than can possibly give rise to mature nuts. The number which develop is a matter of individual difference between the trees; some regularly bear as many as ten, others never more than three. My observation of mature trees has not shown that the drought exercises any influence on the number which blast. It has seemed to me that in a grove in which the trees are in the first year or two of bearing a somewhat larger proportion than usual was blasting during the drought, but then it is also true that a very large percentage always do blast on such trees (on the first branches no nuts mature), so that this effect is uncertain. Neither have I been able positively to determine that the drought exerted any influence on the rapidity of the ripening of the nuts. If there is such an influence it will be toward a more rapid ripening, the tree thus producing smaller nuts, with less store of food. The records of the San Ramon farm show the number of nuts *cut* and the number of nuts and amount of copra sold, but they do not show how many nuts have been picked up from the ground nor at what times nuts have been used for seed; and these items are so considerable that I can draw no sufficiently accurate conclusions as to the yield of copra per nut.[26]

The direct result of the checking of the growth of the young leaves and flowering branches will be a deficiency in the yield of nuts, beginning not less than nine months after the drought first makes itself felt (nine months being about the minimum time between pollination and maturity) and ending at least eighteen months after the drought is broken (that being the usual time elapsing between the appearance of a leaf and the maturing of the subtended nuts).

There are other considerations which make it necessary to extend this period of depression in both directions. When more than a minimum number of nuts are borne on a branch the latter itself is unable to sustain the weight, so that the additional support must be furnished by the petioles of the lower leaves. The untimely casting or depression of these leaves withdraws this support and leaves the branches carrying the greatest load in a condition in which breakage is likely to occur. The nuts

[26] Judging by the eye alone, I can say positively that the nuts cut during April, 1905, averaged distinctly smaller than usual.

are heaviest about three months before maturity, and the loss by premature falling becomes considerable within five months after a drought first becomes serious. At about the same time the drought makes itself felt in injury to the crop through another channel. At all times some nuts are destroyed by crows, but the loss is usually inconsiderable. However, in a period of drought, when other food is scarce and the water courses are dry, they concentrate their attention on the young coconuts and accomplish no little destruction.

The injury to the tree's vitality during a prolonged drought is so severe that the return of favorable weather conditions is but slowly followed by the resumption of the normal activity. When rains come, the roots must awaken from a state of defensive rest in which a prompt response can not be expected. The partly folded condition of the pinnæ, induced by the dryness, seems permanently to remain; at any rate, recovery from it is very slow. A tree which through unfavorable conditions has only twenty-five leaves remaining has not the strength, even under the best conditions, at once to return to the formation of new leaves at the rate which is necessary for the maintenance of a head of thirty. Recovery after a drought is a building-up process, and it must be a slow one. It can hardly be complete in two years, and the return to the normal crop of ripe nuts which can be produced during uninterrupted good seasons can only be well under way in this time.

There is no record of the rainfall at San Ramon prior to September, 1903. The beginning of that year was a period of drought, like the one which has characterized the early months of 1905, but the former can not have been as intense as the latter, for the springs did not so completely disappear. The following record of the number of nuts cut shows how gradually this drought made itself felt and how prolonged its effects have been.[27]

January, 1903	55,160
May, 1903	50,000
August, 1903	45,000
November, 1903	40,723
February, 1904	30,637
May, 1904	33,800
August, 1904	39,765
November, 1904	30,208
February, 1905	31,972
May, 1905	61,550

The report of the superintendent of the farm for June, 1902, states that there were at that time 5,722 coconut trees in bearing on the farm and that 1,809 trees should begin to bear within two years from that date.

[27] The nuts are cut every three months. The work is done by contract, at the rate of 2 pesos per 1,000. This record is made from the "general-expense vouchers" for the expense of cutting.

With this increase in the number of trees, without any improvement in their yields, if there had been no drought, the total cut of nuts for 1904 should have been over 300,000; the actual number was 134,410. The record just given shows that the period of depression which followed the former drought was identical in character with the one to be anticipated from the present condition of the trees. The first real step in return to a fit yield was the cutting of May, 1905, about two years after the former drought ended; the return can not go much farther before the effects of this drought will head it off.

It is then the experience at this farm that a "dry season" occurring only every other year will constantly keep the yield of nuts at considerably less than half of what it would be if the supply of water were always sufficient for the tree's needs. It is obvious that a coconut plantation will be a probable source of continual profit only in localities where dry seasons may never be expected or where it is feasible by irrigation always to keep the ground sufficiently moist to enable the roots to preserve their full, normal activity.

CONCLUSION.

We have just seen that a considerable supply of water must constantly be at the disposal of the coconut, or it will protect itself against injurious desiccation by a partial suspense of its vitality. The necessity of this water as the carrier, in solution, of the plant's mineral and nitrogenous raw food has previously been touched upon. I made no direct experiments in the fertilization of the coconut, but it is the unanimous experience of those who are acquainted with the subject that an increase in some of the constituents of its mineral food has a very marked favorable effect on the production of the fruit.[28] At San Ramon certain trees are pointed out as particularly productive because they have long received the waste from the kitchen. The quantity of mineral food which the tree takes is roughly proportional to the amount of water which it absorbs.[29] Increasing the plant's transpiration has, then, the same effect

[28] Experiments with the object of determining whether the soil surrounding the coconut roots contains nitrifying organisms were undertaken by Dr. W. B. Wherry, of this Bureau. Unfortunately Dr. Wherry left Manila before the work could be completed. Indications of nitrification were not lacking in his work, which is sufficiently encouraging to be continued. The assistance of nitrifying organisms would be a material advantage to the coconut, although it has been shown above that the amount of water which the tree takes up and transpires would, even in such poor soil as that encountered along the beach, contain a sufficient quantity of inorganic constituents to allow the plant to thrive. P. C. F.

[29] It is true that in a wet soil the food is in more dilute solution than in a dry one, but this is partially compensated for by the selective absorption of nutrient salts from very dilute solutions, the solution absorbed being more concentrated than that in the ground. The more dilute the solution the greater is this selective power.

as applying a fertilizer to the ground. The amount of transpiration can be increased in two ways—by increasing the amount of water at the disposal of the roots and by improving the conditions for its evaporation from the leaves.

In seasons of drought the first method does the plant a double service, for the water which is artificially furnished is not only valuable in itself but also because of the substances dissolved in it. However, during other seasons, irrigation may not merely be useless but even very injurious, for ground too wet does not favor the activity of coconut roots any more than that which is too dry.

We have seen that the transpiration of the coconut is somewhat accelerated by the wind, and greatly so by intense illumination. Therefore, so long as the roots are not in too dry a soil, it is in the plant's interest to be exposed as much as is normally possible to these two agents. On any considerable tract devoted to coconut culture this can be done in but one way—by not planting the trees too close together. I have never seen a grove in which the trees were sufficiently far apart so that, unless other conditions were very unfavorable, the trees around the outside were not much more productive than those in the interior. At San Ramon, a considerable proportion of the trees are planted in double rows, one row along each side of a narrow road. In such a row, which contained no nonbearing trees, I found the yield at one cutting to average 22 nuts to the tree. A row of trees along the well-drained bank of a slough yielded an average of 27 nuts, all trees producing. A single tree standing by itself in the open yielded 55 nuts. In the interior of an old grove, the average for the producing trees was about 11, and in the same situation in a large one on the neighboring hacienda of Talisayan the average for bearing trees was only 8; the individuals in the area where this count was made were as a rule about 18 feet apart, their crowns interlaced freely, producing a rather dense shade, and many trees were without ripe nuts.

I have no doubt that up to a distance of at least 15 meters any increase in the intervals between trees will result in an appreciable advance in the average yield per tree, but by planting beyond the intervals at which the interlacing of roots and of leaves would bring the trees into keen competition for water and light, and would also largely break the wind passing through the crowns, the increase in the yield of nuts for the individual trees would not be commensurate with the area of land in use. In my opinion, the trees in a grove can usually best be placed at intervals of about 9 meters. In exposed rows they may well be closer together, and where intense cultivation is economically possible the distance between them may be a little less.

The natural habitat of the coconut is the strand. It is restricted to this because it bears fruit too large to be practically transportable by any

other natural agent than the water; and it is adapted thereto by possessing superficial roots which are uninjured by temporary exposure to concentrated solutions, by having a tough, very elastic trunk, and by producing leaves which are not merely tolerant of the most intense insolation and wind but which are unable to work to the best purpose without more light and wind than many plants can endure. As is true for every cultivated plant, it is possible to create for the coconut conditions altogether more favorable for its utmost thrift than are ever known to occur in nature. It naturally grows in a "poor" soil—that is, in one in which its mineral and nitrogenous raw food is present in very dilute solution. We can improve its environment in this respect, and can profitably carry this improvement much further than is the general practice at present. But the coconut must not be expected to thrive, even in the richest soil and with the best cultivation, if its supply of light is restricted by other trees or in any other way, or where the air is too still or an adequate supply of water is not always available near the surface of the ground.

There is another method of increasing the yield of coconuts, slower but more permanent than improved cultivation; this is by the selection of seed. I have done nothing with this subject, and only mention it because the results of selection can not appear for many years, and a mistaken method would be long in showing its uselessness. Nuts obviously should be selected for seed from trees conspicuous for the amount or quality of their yield. It is usually not a difficult matter to decide whether or not the tree's superior yield is due to its growing under exceptionally favorable conditions. If it is, it shows how other trees may be made to bear equally well, but there is no reason for selecting the nuts of such a tree for seed; its offspring can not be expected to bear more nuts under ordinary conditions than the parent would have done without its exceptional advantages. The environment is not hereditary. The tree the nuts of which should be used as seed is the one the production of which is great in proportion to its opportunity. A tree bearing regularly 12 nuts to the cutting under conditions which allow its neighbors but 8 should have its nuts saved for seed in preference to those of an individual having 30 nuts among equally productive neighbors.

Hygrometer readings, San Ramon farm.

NOVEMBER, 1904.

Date.	Beach.									Rain-fall to 4 p. m.
	6 a. m.			12 m.			4 p. m.			
	Dry.	Wet.	Relative humid-ity.	Dry.	Wet.	Relative humid-ity.	Dry.	Wet.	Relative humid-ity.	
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*	*mm.*
15							25	23.6	89	27
16	23.6	23.1	95	24.1	23	91	23.1	22.9	98	16
17	23.9	22.1	85+	25.5	23.5	85	24.9	23.8	91	3.5
18	24.5	23.7	93	26	29.5	88	26.3	24.6	87	29
19	24.2	23.8	97	27.5	26.6	95	26.2	24.2	85	7
20	25	23.8	91–	29.1	25.5	75	29.1	26.4	80	Trace.
21	24.8	23.2	91–	29.8	27.3	82	28.5	26.3	84	Trace.
22	24.5	23.4	91	28.9	26.1	80	28.6	25.7	79	Trace.
23	27.9	25.4	81	28.6	25.4	77	24.3	23.4	93–	9
24	24.5	23.5	91–	29.7	25.7	72	26	24.5	88	0
25	24.6	21.6	78	23.6	24.6	72	28	24.6	76	0
26	25	23.4	87	29	23.3	62	29.6	24.1	63	0
27	23	21.2	84				27.5	24.2	76	0
28	21.2	20.2	91							
30							27.9	24.8	79	0
Average			88.8			79.9			83.3	91.5

Date.	Interior.								
	6 a. m.			12 m.			4 p. m.		
	Dry.	Wet.	Relative humid-ity.	Dry.	Wet.	Relative humid-ity.	Dry.	Wet.	Relative humid-ity.
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*
15							24.5	23.3	90+
16	23	22.5	95+	23.2	22.9	98	23	22.9	99
17	23.9	22.5	87	25.3	23.1	83	24.5	23.3	91–
18	23.5	23.2	98	24.5	23.5	92	25	24.2	93
19	24	23.5	96	28.5	25	75	25.5	24.5	91
20	27.5	23.4	91–	29.4	25.5	73	28	25.5	82
21	24.3	23	89	28.4	25.7	80	27.5	25.2	83
22	22.5	22	95	28.3	25.7	80	28.4	25.6	80
23	26.9	24.5	81	29	26	78	23.5	22.8	94
24	24.5	23.4	91	28.9	25.4	76	25.9	24.5	89
25	23.9	22.6	89	29	24.5	69+	26.2	24.4	85
26	23.8	23	93	28.5	24	69	28	23.5	69
27	21.8	20.8	91	29.4	25		27.2	24.5	80
28	21	20.2	93						
30							27.8	25.2	81
Average			91.5			81.2			87.6

Hygrometer readings, San Ramon farm—Continued.

NOVEMBER, 1904—Continued.

Temperature and humidity.	Averages.		
	Beach.	Interior.	Difference.
Temperature:	°	°	°
6 a. m	24.3	23.7	−0.6
12 m	28	27.5	− .5
4 p. m	26.8	26	− .8
Humidity:	*Per cent.*	*Per cent.*	*Per cent.*
6 a. m	88.8	91.5	+2.7
12 m	79.9	81.2	+1.3
4 p. m	83.3	87.6	+4.3

DECEMBER, 1904.

Date.	Beach.								
	7.30 a. m.			11.30 a. m.			4 p. m.		
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*
1	22.6	21.6	92	26.4	24.5	85	27.9	24.6	76
2	25	22.3	79	29	24	66	26.2	23.9	83
3	27.2	23.6	74	28.5	24	69	27.6	23.5	72
4	24.3	22.7	87				28	24.2	73
5	25.5	22.6	78	28.8	23.7	65	29.1	24.5	68
6	27.4	24.1	76	27	24.3	80	28	25.1	79
7	26.4	24.4	85	28.3	25.5	80	27.2	25	84
8	25.1	23.2	85	28	26	86	28.3	24.8	75
9	25.3	24.1	91	28.9	25.9	78	27.8	25.5	83
10	26.9	24.5	81	28.5	26.3	84	27.6	25.5	85
11							27.3	25.3	85
12	26.2	24	83	29	26	79	28.1	25.5	81
13	26.4	23.7	79	27.7	24.8	79	28.5	25.5	78
14	26.2	24.8	85						
15	26.8	25.7	92	27.7	25.5	84	28	25.6	83
16	26.5	24.7	86	28.1	26.1	82	29.5	27.2	84
17	25.7	23.9	86	29.1	25.3	73	28.9	25.5	76
18	26.5	23.9	81				28.2	26.3	87
19	25	23.6	89	28.6	25.3	77	27.5	24.8	80
20	25.5	24.4	91	28.3	25.2	78	26.3	24	83
21	24	22.7	89	27.5	25.4	85	27.2	25	84
22	25.3	23.7	87	27.5	24.2	76	27.7	25	78
23	25.9	23.7	83	28	25.5	82	28.5	25.9	81
24	25.7	24	87	27.1	24.8	83	26.6	24.6	85
25	25.6	24.2	89	27.7	24.8	79	26.9	24.7	83
26	24.4	22	80						
27	26.3	24.6	87	27.4	25.3	85	26.8	25.4	89
28	26.7	24.3	82	27.3	25.1	84	27.6	25	81
29	25	22.5	80						
30	26.5	24	81	28.5	25.1	76	28.1	25	78
31	26.2	24	83	27.2	25.2	85			
Average			84.27			79.2			80.52

Hygrometer readings, San Ramon farm—Continued.

DECEMBER, 1904—Continued.

Date.	Interior.									
	7.30 a. m.			11.30 a. m.			4 p. m.			Rainfall.
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*	*mm.*
1	22.5	21.9	95	26.5	24.6	85	26.4	25	89	Trace.
2	25	22.8	83	28.7	25.4	77	26.5	24.7	86	0
3	28.3	25.2	77	28.7	25.6	78	27	23.5	74	0
4	24.3	23	89				27.2	24.2	78	0
5	25.5	22.5	77	28	24	72	28.1	24.1	72	0
6	28	24.4	74	27.9	25	79	27.2	23.9	83	Trace.
7	26.9	24.5	82	28	25.8	84	26.8	25	86	Trace.
8	25.9	24.2	87	28.6	26.1	82	27.5	25.1	8[illegible]	0
9	24.9	24	93	30.6	27.5	78	27.5	25.7	87	Trace.
10	26.3	24.5	86	27.9	25	86	27.5	25	82	Trace.
11							27	25	85	0
12	26.1	24	84	27.9	25	79	27.6	25.4	84	0
13	26.3	24	83	28	24.3	74	27.9	24.9	78	0
14	26.5	24.6	85							0
15	25.4	23.5	85	27.6	25.4	84	27.5	25	82	1
16	28	25.6	83	27.6	25	81	28.3	25.7	81	Trace.
17	25.3	23.9	89	27.8	24.5	76	28.1	25	78	0
18	26.6	24.2	81				27.6	25.5	87	Trace.
19	25.8	24.2	87	27.6	25	81	27.5	24.3	77	Trace.
20	25.5	24.6	93	28.5	25.6	79	26.2	24.3	85	1
21	23.7	23	94	28.4	25.4	78	27.3	24.8	82	Trace.
22	24.8	23.3	88	27.6	24.2	76	27.4	24.7	80	Trace.
23	26.5	24	81	27.5	25.2	84	27.4	24.8	81	0
24	25.1	23.3	86	27.2	24.2	78	26.2	24.4	86	0.5
25	25.1	24	91	27.5	24.5	78	26.5	24.5	85	0
26	24.1	23.1	91							0
27	26.8	25	86	27.2	25.1	85	26.7	24.6	84	0
28	26.8	24.5	83	27.9	25.3	81	27.6	24.4	77	0
29	25.5	22.9	80							0
30	27.7	24.1	74	29.6	25	69	27.5	24.5	78	0
31	27.1	24.9	83	28.5	25.5	78				0
Average			85			79.28			81.85	2.5

Hygrometer readings, San Ramon farm—Continued.

JANUARY, 1905.

Date.	Beach.								
	7.30 a. m.			11.30 a. m.			4 p. m.		
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*
1									
2									
3	26	24.1	85	29.4	24.2	65	27.5	24.5	78
4	26	24.3	87	28.6	24.5	71	26.6	24	80
5	24.8	23	86	27.5	23.9	74	27.1	24	77
6	27.6	24.2	76	27.5	25	82	27.1	24.8	83
7	25.7	23.2	81	28	23.6	69	27.5	23.8	73
8	26	24.4	87				27	24.6	82
9	24.7	23	87	28.3	24.6	74	26.5	24.4	84
10	25.5	23.7	86	27.5	24.3	77	27.5	25	82
11	25.4	23.8	87	28.3	25.1	77	26.5	24.5	85
12	26.8	24	79	29.7	26	74	26.3	24.5	86
13	22.9	22.5	97	27.6	24.9	80	27.3	25.1	84
14									
15									
16				29.1	24.6	70	27	24.5	82
17	24.4	21.9	80	27.6	24.6	78	27.8	24.2	74
18	24.1	22.5	87	27.4	24	75	28	24.5	75
19	25.1	23.4	87	28.8	24	67	28	24.7	76
20	24.3	23.1	91	28	25.5	82	28.8	25.8	75
21	25.4	23.3	84	28	25.8	84	27	24.8	84
22	27.6	21.8	80				27.5	24.7	80
23				28.8	25.9	79	27.2	25.2	85
24	25.1	23.5	87	27.5	25	82	27.6	25.3	84
25	26	24.1	85	28	25.3	80	28.5	25.8	80
26	25.2	23.4	86	28.7	25.9	80	26.8	24.8	85
27	24.5	21.8	79	28.9	25.1	73	27.8	25.1	80
28	24.5	22.5	85	29.4	25.8	75	28.9	25.6	77
29									
30	25	22.5	80						
31				28	24	72	28.2	24.8	76
Average			84.74			75.65			80.28

Hygrometer readings, San Ramon farm—Continued.

JANUARY, 1905—Continued.

Date.	Interior.									Rainfall.
	7.30 a. m.			11.30 a. m.			4 p. m.			
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*	*mm.*
1										0
2										0
3	26.9	24.4	81	29	24	66	27.3	24.1	77	Trace.
4	26.5	23.9	81	29	24.2	68	28	24.7	76	0
5	26	23.5	81	28	23.6	69	26.5	23.6	78	0
6	27.9	24.5	76	28.2	25	77	26.5	24.4	84	0
7	26.5	23.7	79	28.5	23.8	68	27.2	23.6	74	0
8	26.2	24.5	87				26.6	24.2	82	0
9	25.2	23.6	87	28	24	72	26.5	24.3	83	0
10	25.5	23.6	85	28.5	25.4	78	27.5	24.8	80	0
11	25.9	24.2	87	27.7	24.7	78	26.1	24.3	86	0
12	27.5	24.6	79	29.4	25.7	74	26.5	24.3	87	2
13	23.1	22.8	98	27	24.4	81	26.9	24.7	83	26
14										0
15										0
16				29.4	25.4	72	26.5	24.5	85	0
17	25	22.6	81	27.5	24.6	79	27.3	24.1	77	0
18	24.4	22.9	88	27.5	24	75	27.3	24.2	78	0
19	24.7	23.2	88	28.8	23.7	68	27.3	24.3	78	0
20	24.3	23.3	92	28.5	25.5	78	28	25	78	0
21	25	23.3	87	29	26.3	80	27	25	85	0
22	27.7	24.7	78				27.5	25.5	86	2
23				27.4	24.6	80	27.1	25.1	85	0
24	24.9	23.5	89	28	25	78	28	25.4	81	0
25	26.4	24.4	85	28.5	25.5	79	27.7	25	80	Trace.
26	25.6	24	87	28.6	25.6	79	27	25.2	80	0
27	24.7	22	79	28.7	24.9	73	27.5	24.5	78	0
28	25.6	22.5	77	29	25.4	75	28.3	25.2	78	0
29										
30	26.3	24	88							0
31				28	23.7	70	27.6	24.3	76	0
Average			84.13			74.65			80.60	30

Hygrometer readings, San Ramon farm—**Continued.**

FEBRUARY, 1905.

Date.	7.30 a. m.			11.30 a. m.			4 p. m.			Rainfall.
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*	*mm.*
1	24.6	23	87	27.2	24.9	84	28.5	25	75	1
2	24.8	22.7	83							0
3	24.4	22.1	82	27.6	23.5	72	28.5	24.3	71	0
4	24	22.4	87	29.3	25	71	27	23.5	74	0
5	23.9	21.4	80							0
6	24.4	22.6	86	28.6	25	75	28.5	24.9	75	Trace.
7	24.1	22.5	87	29	25.3	74	28	24.5	74	Trace.
8	23.1	21.7	88	28.7	25	74	29	25.4	75	0
9	24	22.1	85	28.8	24.8	72				0
10	21.5	20.5	91	27.2	23.5	73	28.8	24.3	69	0
11	23	21.8	90	27.8	25.1	80				0
12										0
13	24.5	22.6	85	29	25	72	28	24.9	78	0
14	23	21	84	28.1	24.5	75	28.8	24.2	68	0
15	24	21.8	82	28	23.7	70	28.3	23.9	69	0
16	22	20.8	90	28.3	24.4	73	28.6	24.4	71	0
17	23.6	21.9	87	28.4	23.4	66	28	23.7	70	0
18	24.3	22	82	28.4	24	69				0
19										0
20	24.1	22.1	84	28.1	24	71	27.8	24	73	0
21	22.4	20.6	85	28.2	24.5	74	28.4	24	69	0
22	22.6	20.9	87	27.4	24	76	28.4	24	69	0
23	18	16.3	85	26.8	21	59	27.5	23.1	69	0
24	20.4	17.5	75	26.8	22.2	68	27.6	22.6	65	0
25	22.1	20.4	87	28.5	24.5	72				0
26							27.3	23.5	72	0
27	19.8	16.2	69	27.5	23.5	72	27.4	23.2	70	0
28	24.2	21.2	76	28.6	24.5	71	27.8	24	72	0
Average			84.16			72.35			71.4	1

Hygrometer readings, San Ramon farm—Continued.

MARCH, 1905.

Date.	7.30 a. m.			11.30 a. m.			4 p. m.			Rainfall.
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*	*mm.*
1	22.4	20.4	84	28.8	24	70	28	24.5	75	0
2	22	19.5	79	27.8	23.5	70	28.5	25	75	0
3	24.3	22	82	28.4	24.6	73	27.5	24.1	75	0
4	23.1	21	83	28	24.6	76				0
5										0
6	23.5	20.6	77							0
7				29.1	24.6	69	27.5	24.3	77	0
8	23.6	22	87	29	24.9	72	26.9	24.4	81	0
9	24.8	23	86	28.2	23.6	68	26.8	23.5	75	0
10	24	21.2	78	29	23.6	63	27.9	24	72	0
11	23.2	21.2	84	28.7	24.2	69	27.5	23.5	71	0
12	24.5	21.7	78	28.2	24.6	74	27.4	24.6	80	0
13	24	22.1	85	28.5	25.5	78				0
14	25.2	23.3	85	28.6	26	81	28.9	25.5	76	0
15	25	22	77	29	25.4	75	28.6	25.5	78	0
16	25	23	84	29.1	25.8	77	28.7	25	74	0
17	25.5	23.5	84	29	26	79	28	25.4	81	Trace.
18	24.7	22.9	86	28.6	26	81				0
19	25.8	23.8	85	29.8	26.3	76	29	26.1	79	0
20	26.1	23.8	83	29.5	26.4	80	27.9	25.7	84	0
21	24.5	22.5	84	29.5	26.5	79	28.9	26.1	80	0
22	26	23.4	80	29.5	27	82	29.2	25.9	77	0
23	26.5	23.9	80	29.1	26.8	80				0
24	25	22.8	83	29.3	26.5	80	29.8	26.4	77	0
25	26.2	23.3	78	29	25.9	78	28.3	25.8	82	0
26										0
27	26.7	24	79	29.7	27.1	81	29.7	26.5	78	0
28	27	24	78	29.6	26	75				0
29	26.2	23.6	81	31	27	73	29.8	26.3	77	0
30	27.4	24.6	80	29.3	26	77	29.8	26.1	76	Trace.
31	26	24.1	85	29.6	26.3	77	30	26.8	78	0
Average			81.96			75.46			77.30	0

Hygrometer readings, San Ramon farm—Continued.

APRIL, 1905.

Date.	7.30 a. m.			11.30 a. m.			4 p. m.			Rain-fall.
	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	Dry.	Wet.	Relative humidity.	
	°	°	*Per cent.*	°	°	*Per cent.*	°	°	*Per cent.*	*mm.*
1	26.4	24.9	88	28.1	25.9	84				0.5
2										0
3				28.8	26.2	81	29	26	79	Trace.
4	27.1	24.5	81	31	27.6	77	30	25.9	72	0
5	26.9	24.1	79	30.5	25.3	66	29.3	25	70	0
6	25.6	23.8	86	30.6	26.2	70	28.9	25.5	76	0
7	26.6	24.5	84	30	25.9	72	28.9	25.3	75	Trace.
8	26.7	24.6	84	28.8	26	80	30.5	26.9	76	0
9	26.9	25.1	86	29.4	26.4	79	28.6	25.4	77	0
10	25	23.4	87	30.6	26.6	73	29.9	26.1	73	Trace.
11	25.2	23.2	85	28.9	26.2	80	28	26	85	3
12	24.7	23.6	91	28.9	26.1	80	29.4	26.5	80	Trace.
13	25.8	23.9	85	28.7	26.2	82	26	24.4	87	9
14										Trace.
15	26.1	23.8	82	29.3	26.4	79	30.7	26.2	69	0
16	26.7	23.8	77							0
17										0
18										0
19	27.2	24.3	78	29.4	26	75	28.6	25.5	77	0
20	26.5	24.1	81	30	26.8	77	28.8	26	79	0
21	27.2	25	83	29.8	25.9	72	30	26	71	0
22	27.7	24.9	79	29.6	26	74	29.3	26.2	77	0
23	26.3	24.2	83	30.6	26.2	70	32	26.7	64	0
24	26.8	23.3	73	30.7	26.1	68	29.9	25.9	71	0
25	27.2	24.3	78	31.2	27.4	73	30	26.6	75	0
26	26.6	23.9	79	30.1	26.3	73	30.4	26.3	71	0
27	27.3	24.8	81	31.1	26.6	69	29	26.7	83	0
28	26.9	24.1	78							0
29	26.6	23.7	77	32.2	27.3	67	32.3	27.9	70	0
30										
Average			81.875			74.9			75.32	12.5

ILLUSTRATIONS.

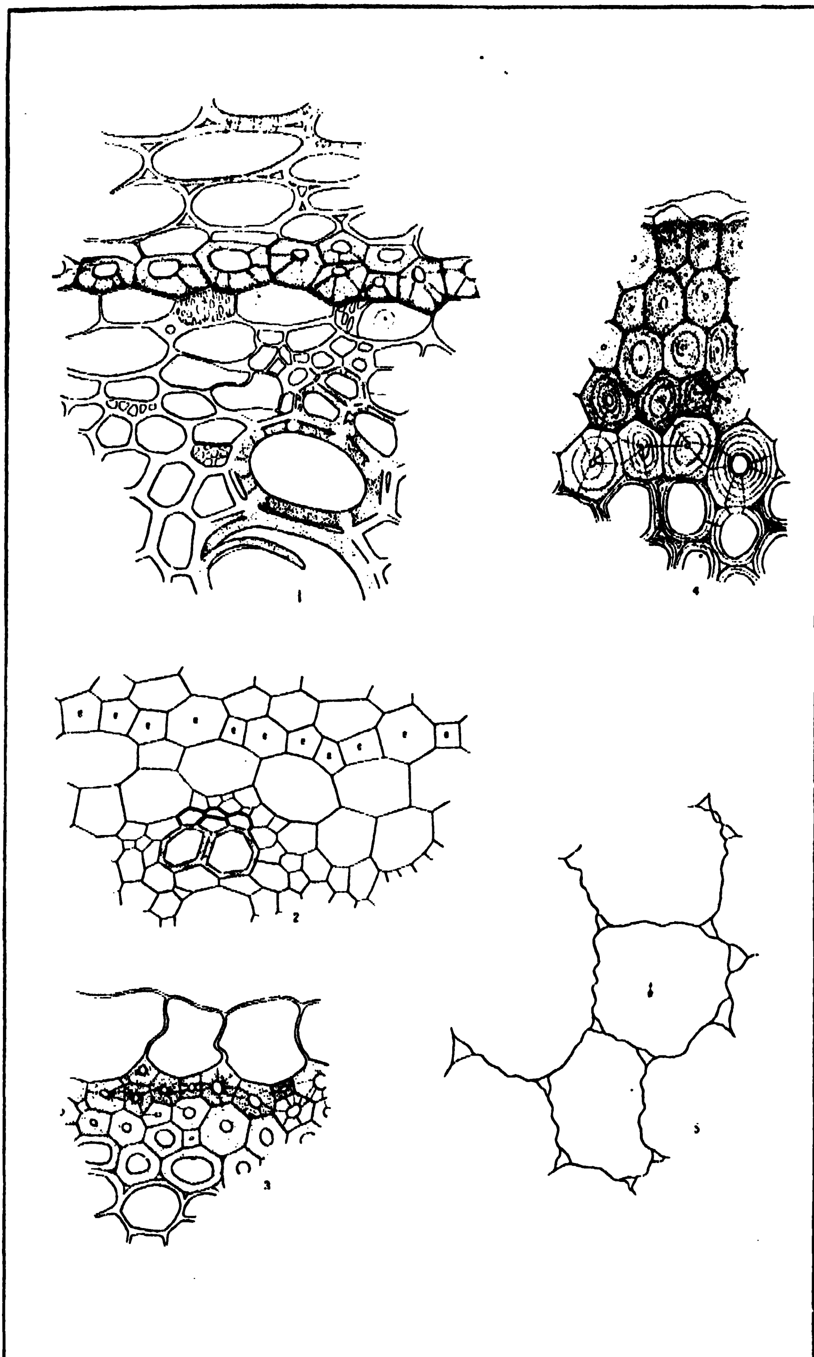

PLATE I.

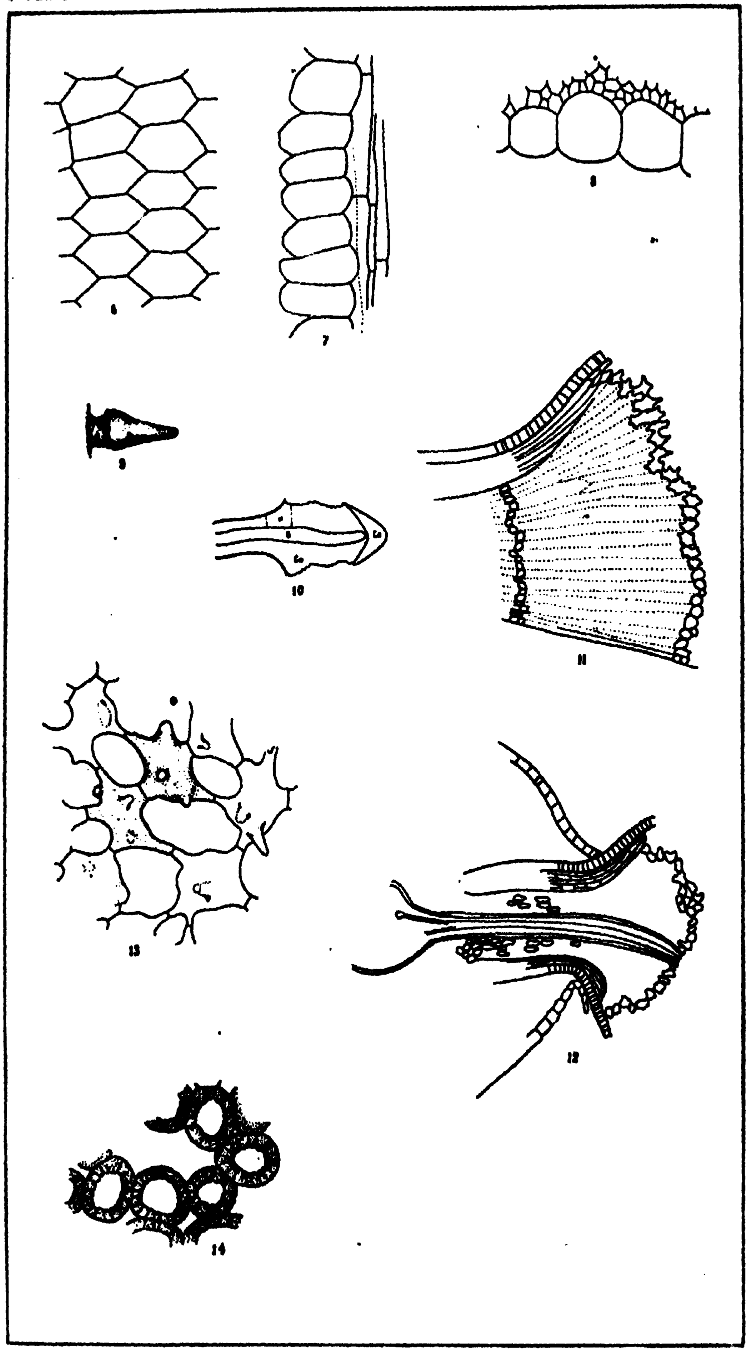

PLATE II.

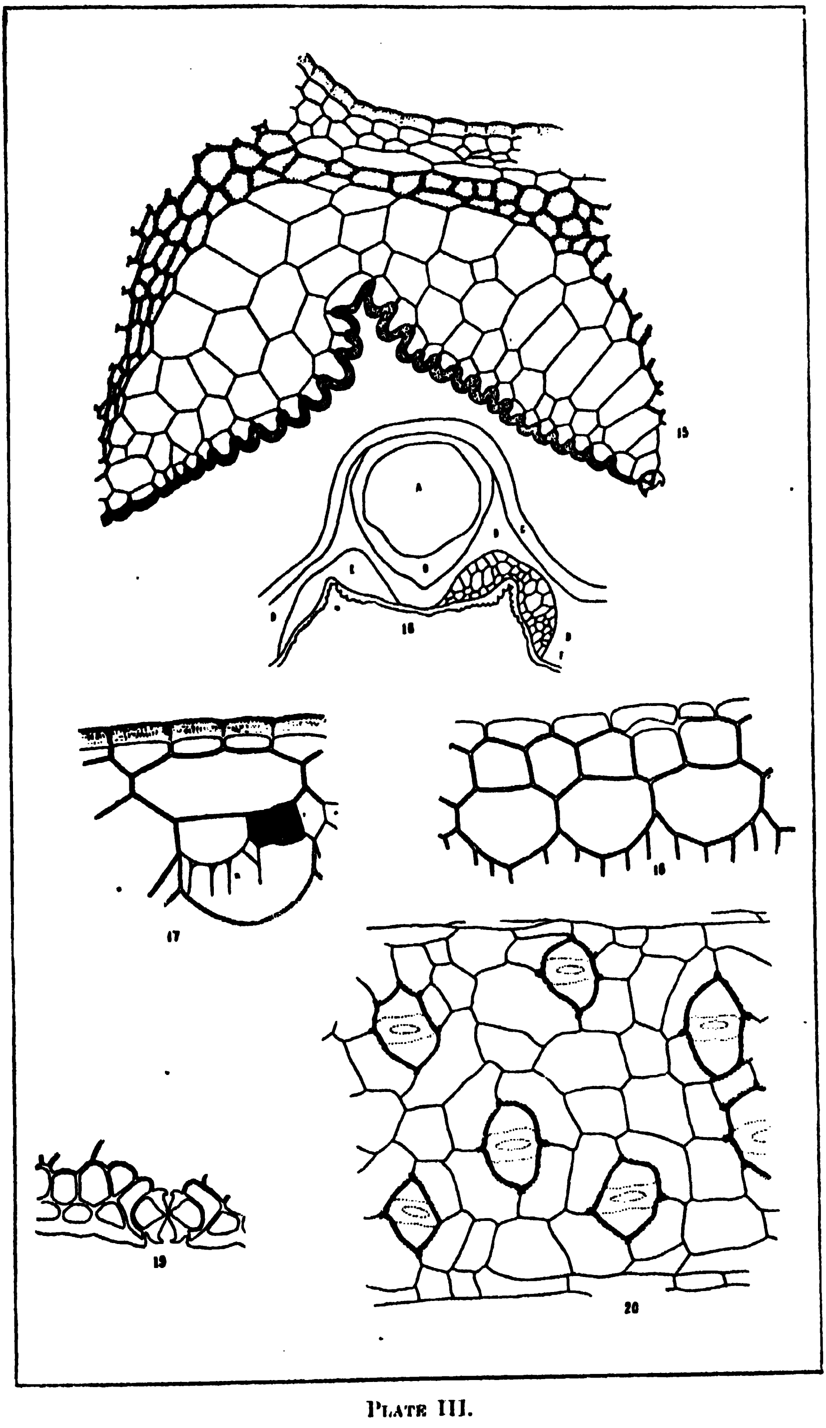

PLATE III.

THE COCONUT AND ITS RELATION TO THE PRODUCTION OF COCONUT OIL.

By Herbert S. Walker.
(From the Chemical Laboratory, Bureau of Science.)

THE SOIL.

Coconut production from the standpoint of the quality and quantity of the oil yielded has hitherto not been investigated, and it was decided to enter into this subject as fully as possible. The first problem which presented itself was the influence of the soil in which the trees grow on the yield of nuts, copra, and oil. It had been noticed for a long time that coconut trees growing near the seashore at San Ramon produced much more fruit than those standing farther inland, and it had also been stated that the former trees bear a better quality of nuts than the latter.

To determine how far these facts might be accounted for by a greater fertility of the soil near the sea, the following analyses were made of a number of soils in which coconut trees were growing, the samples being taken at the beach as well as farther inland, and two from Davao, where coconut trees flourish:

Analyses of San Ramon soils.

Sample.	Fine earth.	Moisture.	Loss on ignition.	P_2O_5.	K_2O.	N.	Fine earth, Cl.	Coarse earth, Cl.
	60 mesh.							
A_1	22.5	2.65	5.83	0.08	0.36	0.18	0.012	0.001
A_2	7	2.45	3.06	.07	.52	.03	.018	.002
A_3	23	1.99	1.68	.07	.55	.02	.009	.002
B_1	52	5.11	7.93	.33	.58	.13	.018	.001
B_2	37.5	7.82	5.97	.08	.45	.04	.004	.001
B_3	45	7.33	6.03	.08	.48	.03	.006	.001
C_2	3.7	2.55	1.53	.10	.88	.003		
C_3	2.1	2.65	1.45			.02		
	30 mesh.							
D_1	38	2.52	1.35	.11	.18	.01		
D_2	26.9	2.96	1.71	.11	.62	.004		
D_3	37.2	2.32	2.29	.07	.65	.01		
	40 mesh.							
E	43.6	7.6	1.79	.08	.40	.05		
F	85.7	24	6.04	.24	.21	.11		

Moisture on samples E and F determined at San Ramon; on all others at the laboratory, Manila.

Fine earth determined on original samples.

Loss on N, P_2O_5, K_2O determined and percentages calculated to original sample on soil dried at 100°–105°.

Soils marked "A" were taken at a distance of 60 feet from the sea, A_1 being the surface, A_2 18 inches, and A_3 3 feet below.

The B soils were taken from 2,800 feet inland, 40 feet above sea level, where trees were not bearing so well, and at the same depths as the A soils of corresponding numbers.

The C soils were from the same place as those marked "A," but were taken at a greater depth so as to reach the locality of the deepest roots, C_2 being from 4 feet and C_3 from 8 feet below the surface.

The D soils were taken from the same place and depths as the C soils.

Soil E was taken at a depth of 3 to 4 feet, 6 feet distant from a very healthy 5-year-old tree near the sea.

Soil F was taken at a depth of about 3 feet and about 1,800 feet from the sea, where trees do not bear so well.

Davao soils.

Sample.	Fine earth.	Moisture.	Loss on ignition.	P_2O_5.	K_2O.	N.	CaO.
	40 mesh.						
I	95	7.60	5.42	0.16	0.26	0.05	2.85
II	91.9	1.30	1.42	.11	.13	.03	2.06

Soil marked "I" was taken at a distance of 50 yards from the Davao River about 1 mile inland from the sea, where trees were growing well.

Soil marked "II" was taken at the mouth of the Davao River about 50 feet from the sea. In this location a few young trees were doing fairly well.

Both samples were taken at a depth of about 1 foot.

Chemically, the results of these analyses show very little difference between the soils near the shore and those farther inland. The latter, contrary to what would be supposed, were found to be somewhat superior to the former, although neither could be called extremely fertile. Chlorine was determined in the first six of these samples, with the idea that this element might play some part in the better growth of trees near the sea, but the amounts found were so small as to be almost negligible. From these results it is evident that the inferior quality of the inland trees can not be explained by the analytical difference in the soils; neither does the salt from the sea appear to an appreciable extent, even around those trees which are actually growing on the beach.

However, the superior growth of trees near the sea might well be accounted for theoretically by the physical characteristics of the soil alone. For example, the soil marked "E" in the foregoing table is practically nothing but a very porous sand which, at a depth of 3 feet, is completely saturated with moisture; while F is a very stiff clay, such as the Spaniards formerly used for making bricks. While it is true that the latter contains more total moisture and plant nutriment than the

former, the amount available to the tree is probably not by any means as great, owing to the difference in porosity.[1] In view of the large amount of water necessary to the life of the coconut tree, one would naturally expect it to grow better in an easily permeable soil rather than in one from which water and soluble nutriment can only be taken up with difficulty. The objection has been raised that according to chemical analyses the soils near the sea do not appear to contain sufficient plant food to support life of any kind, much less that of a large and heavily productive tree like the coconut.

From analyses of coconuts made at this Bureau we have found that nuts from San Ramon contain nitrogen, potash, and phosphoric acid in approximately the following amounts:

Part.	Nitrogen.	Potash.	Phosphoric acid.
	Grams.	*Grams.*	*Grams.*
Husk	1.609	3.915	0.017
Shell	660	.947	.459
Meat	4.683	2.475	1.740
Milk	1.542	1.313	.171
Total	8.494	8.650	2.387

In 1 hectare of land on which about 173 trees are growing and producing, a total of about 7,000 nuts per annum may be expected.[2] Under these conditions there is exhausted from the soil by the nuts alone a total of—

	Kilos.
Nitrogen	59.43
Potash	60.55
Phosphoric acid	16.73

In addition to this there is a large weight of material withdrawn by falling leaves. Each tree on an average will lose annually 16 leaves, weighing about 3 kilos each, making about 8,300 kilos per year lost by 173 trees. Analysis shows that in this weight of dry leaves there is approximately—

	Kilos.
Nitrogen	31.69
Potash	74.82
Phosphoric acid	24.65

We have then a total annual drain on the soil per hectare of—

	Kilos.
Nitrogen	91.12
Potash	135.37
Phosphoric acid	41.38

[1] See the paper by E. B. Copeland on the character of the roots of the *Cocos*.

[2] This is on the basis of 40 nuts per tree per annum, a very high average for San Ramon trees.

Assuming these figures to be approximately correct, it would appear at first glance somewhat of a puzzle to determine how the tree manages to thrive and take up so much nourishment each year from a soil seemingly so devoid of fertility as that along the sea at San Ramon. However, when we consider the total amount of soil available to each tree, the problem becomes a simple one. The root mass of a coconut draws nutriment from a depth of at least 2 meters below the surface of the ground and outward on all sides for from 3½ to 6½ meters distance from its base. It thus comes in contact with an exceedingly large mass of material and it makes use of all the available nourishment therein.

In 1 hectare, or 10,000 square meters, of land there is available to the coconut trees planted thereon a total of at least 20,000 cubic meters of soil, or, if we allow a specific gravity of about two, 40,000,000 kilos.

From the table of analyses of San Ramon soils, we find that the soils near the sea average about as follows:

	Per cent.
Nitrogen	0.07
Potash	.50
Phosphoric acid	.07

In 40,000,000 kilos we have—

	Kilos.
Nitrogen	28,000
Potash	200,000
Phosphoric acid	28,000

From the amount taken from the soil in each year, even though no fresh addition were made, we can calculate the number of years required completely to exhaust this soil of its plant food as follows:

	Years.
Nitrogen	307
Potash	1,478
Phosphoric acid	677

These figures are naturally only an approximation, but they show that even in a comparatively poor ground there exists more than an abundance of nourishment for the coconut tree, provided the soil itself is sufficiently porous and well watered.[3]

It seems very probable that in San Ramon at least, if not in most plantations along the seacoast, the nutritive material comes not from the soil in which the trees are actually growing but from an inexhaustible supply of water, laden with plant food, which is constantly seeping down from the higher ground toward the ocean. This underground water supply would account for the flourishing condition of trees in a sandy soil near the sea, even in times of drought, when individuals farther inland in higher, less permeable ground would be dying from want of water.

[3] See the paper by E. B. Copeland on the transpiration of the coconut and the amount of water taken up by an individual tree.

Fertilization and irrigation.—In the case of the less permeable soils, artificial irrigation during the dry season would seem to be of the utmost importance, and any addition to the fertility of the land, either in the form of manure or of a chemical fertilizer, would probably be repaid by an increased yield of fruit. For soils near the sea, under conditions such as exist at San Ramon, irrigation is of course unnecessary excepting in times of extreme drought, and fertilization would be of doubtful advantage, as the trees in such a location seem to be growing under the best conditions possible without any attention whatsoever. Fertilizing material in such localities would probably be leached out and carried into the sea before it could be of much value to the trees.

THE NUT AND ITS OIL PRODUCTION.

The analytical methods used in compiling the accompanying tables were as follows: The weights, in grams, of husk, nut minus husk, shell, and milk were determined directly. To avoid loss by evaporation the meat itself was not weighed but was assumed to be the difference between the weight of the whole nut (minus the husk) and the combined weights of shell and milk.

Copra.—The meat from each nut was allowed to dry in the air over night, so as to assume a fairly constant weight, and was then weighed directly; 25 grams were then cut into fine pieces and dried to constant weight at 100° C. for the determination of anhydrous copra, the latter being calculated back to per cent in the fresh meat. To approximate the amount of commercial copra obtainable, an addition of about 10 per cent should be made because of the water ordinarily contained in this product.

Oil.—The anhydrous copra prepared at San Ramon was sealed in glass bottles and shipped to Manila for analysis, the majority of the oil determinations being made by Mr. George F. Richmond, of this laboratory. Before this time much work had been done in devising a method for the rapid and accurate estimation of oil in copra. It was found to be almost impossible to make a complete extraction by the ordinary method of cutting fine pieces and extracting with ether in a Soxhlet cone. Even after the apparatus had been running for forty hours, a small increase in weight was obtained by extracting for eight hours more. Grinding with sand and then extracting with ether produced some improvement. Extraction with hot chloroform alone took out a little more oil, but it was necessary to continue the operation for at least sixteen hours. The method finally used was as follows:

A 2-gram sample was intimately ground with fine sand in a glass mortar, the mixture transferred to a Soxhlet cone, the mortar washed two or three times with fresh sand, and then finally wiped with fat-free cotton. The extraction with hot chloroform takes three hours.

The chloroform is then distilled and the remaining oil dried to constant weight at 100° C. Experiment demonstrated that practically all the oil was extracted in two hours. The chloroform extract made in this way proved to be entirely soluble in absolute ether. The sand used was prepared from ordinary sea sand by taking all which went through a 30-mesh and which was retained on a 100-mesh sieve, heating this product for some time to destroy organic matter and then afterwards extracting with chloroform.

Age in reference to quality of the nut.—One of the first and most important of the problems which presented itself was to determine the effect of the age and the relative maturity of the nut on the percentage of its various constituents; in other words, to find out the most favorable time for opening a nut to obtain the largest and best yield of copra and of oil. With this end in view, the following analyses were made of four series of ten nuts each:

Series I consisted of 10 nuts selected from a pile which had just been picked from the trees and which were ready to be used for making copra. These nuts were all fairly ripe, although the husks were still green in color and full of water.

Series II was made up of 10 nuts from the same pile as Series I, but all were very ripe. The husks were dry and of a dead-brown color.

Series III represents nuts which had been selected for seed and set out to sprout about three months before the time of analysis. They each contained a small embryo or "foot" and had green sprouts protruding to a height of about 6 inches. The husks had absorbed a large amount of water while lying on the ground.

Series IV was a rather abnormal lot of nuts which had been set out for seed similarly to those in Series III, but for some reason the individual ones had failed to sprout, although they had been standing for six months. The meat had become somewhat softened and in several cases it was discolored and possessed a bad odor. To a greater extent than any other this series shows what large variations may exist among nuts of the same age.

SERIES I.—*Ten nuts, fresh from trees but fairly ripe (all green husks).*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.			
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1	2,702	1,160	42.9	1,542	57.1	290	10.8	725	26.8	45.2	54.8
2	2,725	1,265	46.4	1,460	53.6	330	12.1	590	21.7	43.9	56.1
3	3,039	1,510	49.7	1,529	50.3	395	13.0	639	21.0	54.8	45.2
4	3,182	1,547	48.6	1,635	51.4	340	10.7	585	18.4	32.8	67.2
5	2,830	1,300	45.9	1,530	54.1	299	10.6	606	21.4	44.5	55.5
6	2,316	1,106	47.8	1,210	52.2	239	10.3	541	23.4	46.1	53.9
7	2,872	1,157	40.3	1,715	59.7	338	11.8	652	22.7	43.9	56.1
8	2,930	1,350	46.1	1,580	53.9	345	11.8	580	19.8	48.5	51.5
9	2,100	925	44.0	1,175	56.0	260	12.4	505	24.1	53.2	46.8
10	3,787	1,915	50.6	1,872	49.4	395	10.4	717	18.9	51.7	48.3
Average	2,848	1,323	46.2	1,525	53.8	323	11.4	614	21.8	46.5	53.5

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	327	67.2	32.8	12.1	527	19.5	220	8.1	18.8	47.0	21.2	34.2	14.3
2	259	66.4	33.6	9.5	540	19.8	172	6.3	22.6	40.4	17.7	37.0	11.8
3	351	69.3	30.7	11.5	495	16.3	243	8.0	25.8	41.8	23.0	32.4	15.9
4	192	59.8	40.2	6.0	710	22.3	115	3.6	20.8	35.8	11.7	43.4	7.0
5	270	63.4	36.6	9.5	625	22.1	171	6.0	19.5	39.6	17.7	40.9	11.2
6	250	64.9	35.1	10.8	430	18.6	162	7.0	19.8	44.7	20.7	35.5	13.4
7	287	62.3	37.7	10.0	725	25.2	179	6.2	19.7	38.0	16.7	42.3	10.4
8	281	63.0	37.0	9.6	655	22.3	177	6.0	21.8	36.7	17.8	41.5	11.2
9	269	65.3	34.7	12.8	410	19.5	176	8.4	22.1	43.0	22.9	34.9	15.0
10	371	68.7	31.3	9.8	760	20.1	255	6.7	21.1	38.3	19.8	40.6	13.6
Average	286	65.0	35.0	10.2	588	20.6	187	6.6	21.2	40.5	18.9	38.3	12.4

SERIES II.—*Ten nuts, very ripe (dead-brown husks).*

[Selected from pile of several thousand.]

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.			
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1	2,616	665	25.4	1,951	74.6	352	13.5	774	29.6	43.5	56.5
2	1,935	350	18.1	1,585	81.9	328	17.0	602	31.1	59.9	40.1
3	2,025	460	22.7	1,565	77.3	262	13.0	644	32.5	50.9	49.1
4	1,681	332	19.7	1,349	80.3	288	16.8	596	35.5	51.5	48.5
5	2,070	480	23.2	1,590	76.8	320	15.5	585	28.2	51.6	48.4
6	2,192	647	29.5	1,545	70.5	380	17.4	715	32.6	56.3	43.7
7	2,945	460	15.6	2,485	84.4	430	14.6	980	33.3	42.4	57.6
8	1,948	437	22.4	1,511	77.6	330	17.0	641	32.9	56.9	43.1
9	2,049	500	24.4	1,549	75.6	309	15.1	675	32.9	51.7	48.3
10	1,735	425	24.5	1,310	75.5	260	15.0	590	34.0	54.4	45.6
Average	2,120	476	22.6	1,644	77.5	326	15.5	680	32.3	52.0	48.0

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	337	54.0	46.0	12.9	825	31.5	182	7.0	18.0	39.7	17.3	42.3	9.3
2	360	66.4	33.6	18.6	655	33.8	239	12.3	20.7	38.0	22.7	41.3	15.1
3	328	61.7	38.3	16.2	659	31.8	202	10.0	16.7	41.2	21.0	42.1	12.9
4	307	62.2	37.8	18.3	470	28.0	191	11.4	21.0	44.2	22.8	34.8	14.2
5	302	59.6	40.4	14.6	685	33.1	180	8.7	20.1	36.8	19.0	43.1	11.3
6	402	58.9	41.1	18.3	450	20.5	237	10.8	24.6	46.3	26.0	29.1	15.3
7	415	58.1	41.9	14.1	[a]1,075	36.5	241	8.2	17.3	39.4	16.7	43.3	9.7
8	365	66.3	33.7	18.7	540	27.7	242	12.4	21.8	42.4	24.2	35.8	16.0
9	349	63.3	36.7	17.0	565	27.6	221	10.8	19.9	43.6	22.5	36.5	14.3
10	321	63.1	36.9	18.5	460	26.5	203	11.7	19.9	45.0	24.5	35.1	15.5
Average	349	61.4	38.6	16.7	638	29.7	214	10.3	20.0	41.7	21.7	38.3	13.4

[a] Milk very turbid.

SERIES III.—*Nuts stored three months, just beginning to sprout.*

No.	Total weight.	Embryo.	Husk.		Nut minus husk.		Shell.		Meat.			
			Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1	2,935	15	1,313	44.7	1,622	55.3	326	11.1	711	24.2	57.2	42.8
2	2,138	25	1,060	49.7	1,078	50.3	232	10.9	558	25.9	50.7	49.3
3	1,966	15	729	37.1	1,237	62.9	275	14.0	557	28.3	55.7	44.3
4	1,666	10	565	33.9	1,101	66.1	242	14.5	509	30.6	52.8	47.2
5	2,791	20	1,653	59.2	1,138	40.8	232	8.3	561	20.1	49.9	50.1
6	2,537	5	1,395	55.0	1,142	45.0	285	11.2	505	19.9	52.8	47.2
7	2,664	50	973	36.5	1,691	63.5	299	11.2	752	28.2	49.9	50.1
8	3,993	5	2,731	68.4	1,262	31.6	281	7.0	536	13.4	51.5	48.5
9	5,062	15	3,115	61.5	1,947	38.5	398	7.9	849	16.8	40.3	59.7
10	2,300	10	1,160	50.4	1,140	49.6	273	11.9	540	23.5	51.7	48.3
Average	2,805	17	1,469	49.6	1,335	50.4	284	10.8	607	23.1	51.3	48.7

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	407	62.4	37.6	13.9	570	19.4	254	8.7	20.1	43.8	25.1	35.2	15.7
2	280	59.5	40.5	13.1	263	12.3	167	7.8	21.6	51.5	26.1	24.5	15.5
3	311	63.8	36.2	15.8	390	19.8	199	10.1	22.2	44.9	25.2	31.5	16.0
4	269	63.6	36.4	16.1	340	20.4	171	10.3	22.0	46.2	24.4	30.9	15.5
5	280	65.1	34.9	10.0	325	11.6	182	6.5	20.4	49.3	24.6	28.6	16.0
6	266	58.7	41.3	10.5	347	13.7	156	6.2	25.0	44.2	23.3	30.4	13.7
7	375	60.0	40.0	14.1	590	22.2	225	8.4	17.7	44.5	22.2	34.9	13.3
8	276	60.6	39.4	6.9	440	11.0	167	4.2	22.2	42.5	21.9	34.9	13.3
9	342	62.2	37.8	6.8	685	13.5	212	4.2	20.4	43.6	17.6	35.2	10.9
10	279	62.1	37.9	12.1	317	13.8	173	7.5	24.0	47.4	24.5	27.8	15.2
Average	309	61.8	38.2	11.9	427	15.8	191	7.4	21.6	45.8	23.5	31.4	14.5

SERIES IV.—*Nuts stored six months which had not sprouted.*

[Most nuts of this age have sprouts 20 to 30 centimeters long.]

No.	Total weight.	Embryo.	Husk.		Nut minus husk.		Shell.		Meat.			
			Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
		Grams.										
1	2,908	6	1,272	43.7	1,636	56.3	367	12.6	683	23.5	51.8	48.7
2	2,541		1,382	54.4	1,159	45.6	197	7.8	492	19.3	33.0	67.0
3	3,419		1,743	51.0	1,676	49.0	360	10.5	666	19.5	44.1	55.9
4	3,154		1,462	46.3	1,692	53.7	277	8.8	800	25.4	51.3	48.7
5	2,023	5	1,123	55.5	900	44.5	252	12.5	500	24.7	54.7	45.3
6	2,276		1,228	54.0	1,048	46.0	160	7.0	478	21.0	33.2	66.8
7	3,116		1,939	62.2	1,176	37.8	260	8.4	536	17.2	37.3	62.7
8	2,403		1,443	60.1	960	39.9	180	7.5	453	18.8	29.3	70.7
9	3,238		1,800	55.6	1,438	44.4	262	8.1	696	21.5	34.9	65.1
10	3,585		2,225	62.1	1,360	37.9	272	7.6	638	17.8	38.2	61.8
Average	2,866		1,562	54.5	1,305	45.5	259	9.1	594	20.9	40.7	59.3

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	350	61.4	38.6	12.1	580	20.1	216	7.4	22.5	41.6	21.4	35.5	13.1
2	170	67.7	32.3	6.7	470	18.5	115	4.5	17.0	42.5	14.7	40.5	9.9
3	294	69.0	31.0	8.6	650	19.0	203	5.9	21.5	39.7	17.5	38.8	12.1
4	410	68.4	31.6	13.0	615	19.5	281	9.9	16.4	47.3	24.2	36.3	16.6
5	274	74.4	25.6	13.5	143	7.1	204	10.1	28.0	55.6	38.3	15.9	22.7
6	136	56.3	43.7	6.0	410	18.0	77	3.4	15.8	45.6	13.0	39.1	7.3
7	200	66.2	33.8	6.4	380	12.2	132	4.2	22.1	45.6	17.0	32.3	11.3
8	132	74.1	25.9	5.5	327	13.6	98	4.1	18.7	47.2	13.8	34.1	10.2
9	242	63.4	36.6	7.5	480	14.8	153	4.7	18.2	48.4	16.8	33.4	10.7
10	244	58.5	41.5	6.8	450	12.5	148	4.0	20.0	46.9	17.9	33.1	10.5
Average	245	65.9	34.1	8.6	451	15.5	162	5.8	20.0	46.0	19.5	34.0	12.4

The variation among individual nuts in the foregoing analyses was rather greater than had been expected, and it is doubtful if even an average of ten nuts gives more than an approximation of their true value at a given age.

However, considering the average percentages as calculated to the nut free from husk, there appears a gradual increase in the proportion of meat, copra, and oil from Series I to Series III, with a corresponding decrease in the percentage of milk, indicating that the meat is becoming firmer, is losing some water and gaining oil, as the nut increases in age. In Series IV, those nuts which had been kept for six months, the meat remains practically the same in amount, but there is a marked drop in the proportion of copra and oil, probably due to decomposition or other changes which are beginning to take place in the meat. However, No. 5 of this series, a nut in which decomposition had already set in, shows an abnormally high percentage of both copra and oil, a fact which is very hard to account for, although it is possible that this individual may have been still higher in these substances before decomposition began. In both Series I and IV the percentage of oil in the anhydrous copra is considerably higher than it is in II and III, though this is more than counterbalanced by a much lower proportion of copra in the meat. Both in very fresh and in overripe nuts there is a considerable deficiency in oil, but the principal loss is in the amount of copra to be obtained, this result being due to a higher percentage of water as compared with solid matter in the meat. In all these nuts it will be noticed that the proportion of shell to the whole nut varies but little.

Analyses of nuts from the same trees but of varying degrees of ripeness.—In order as much as possible to eliminate the variations in the individual nuts, and to discover if those taken from the same tree would not show greater uniformity in their composition, fifty nuts from one tree near San Ramon were procured for analysis.

Ten of the least ripe among these were analyzed as shown in Series V. All of the individuals of this series were well developed externally, but were full of milk, and not yet sufficiently mature to be picked for making copra.

The ten ripest nuts of the lot were next selected (Series VII). Their husks were of a dead-brown color and thoroughly dry.

The remaining thirty were in a condition which might be termed "fairly ripe"—that is, they were of the kind ordinarily used for making copra. Nine of these were analyzed at once (Series VI), and the remainder shipped to Manila for storage and future analysis. In Series V, VI, and VII "total solids" in the milk were determined in addition to the regular analysis.

Series V.—*Ten nuts not fully ripe, fresh from tree.*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.				Copra (anhydrous).	
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.	Weight.	Per cent oil.
1	4,227	2,995	70.9	1,232	29.1	232	5.5	410	9.7	33.2	66.8	136	----
2	4,018	2,770	69.0	1,248	31.0	240	6.0	428	10.6	34.3	65.7	147	66.9
3	4,012	2,882	71.9	1,130	28.1	220	5.5	378	9.4	30.0	70.0	114	63.9
4	4,535	3,380	74.5	1,155	25.5	219	4.8	394	8.7	27.7	72.3	109	64.5
5	3,737	2,655	71.0	1,082	29.0	205	5.5	362	9.7	26.0	74.0	94	56.0
6	3,931	2,787	70.9	1,144	29.1	214	5.5	418	10.6	33.2	66.8	139	62.0
7	3,919	2,850	72.7	1,069	27.3	212	5.4	347	8.9	29.8	70.2	103	64.6
8	3,967	2,700	68.1	1,267	31.9	244	6.1	419	10.6	37.2	62.8	156	66.9
9	4,046	2,806	69.4	1,240	30.6	239	5.9	450	11.1	37.5	62.5	152	65.8
10	3,187	1,965	61.7	1,222	38.3	240	7.5	445	14.0	48.2	51.8	214	69.9
Average	3,958	2,779	70.0	1,179	30.0	227	5.8	405	10.3	33.7	66.3	136	64.5

No.	Copra (anhydrous).		Milk.				Oil.		Calculated to per cent in nut free from husk.				
	Per cent plup.	Per cent copra in nut.	Per cent solids.	Per cent water.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	----	3.2	6.0	94.0	590	13.9	----	----	18.8	33.3	11.0	47.9	----
2	33.1	3.7	5.9	94.1	580	14.4	98	2.4	19.2	34.3	11.8	46.5	7.9
3	36.1	2.8	6.5	93.5	532	13.2	73	1.8	19.5	33.4	10.1	47.1	6.4
4	35.5	2.4	6.5	93.5	542	12.0	70	1.6	19.0	34.1	9.4	46.9	6.1
5	44.0	2.5	6.7	93.3	515	13.8	53	1.4	18.9	33.5	8.7	47.6	4.9
6	38.0	3.5	6.0	94.0	512	13.0	86	2.2	18.7	36.5	12.2	44.8	7.5
7	35.4	2.6	6.6	94.1	510	13.0	67	1.7	19.8	32.6	9.6	47.7	6.2
8	33.1	3.9	6.7	93.3	604	15.2	104	2.6	19.3	33.1	12.3	47.6	8.1
9	34.2	3.8	6.0	94.0	551	13.6	100	2.5	19.3	36.3	12.3	44.4	8.1
10	30.1	6.7	5.6	94.4	537	16.8	150	4.7	19.6	36.4	17.5	44.0	12.2
Average	35.5	3.5	6.3	93.7	547	13.9	89	2.3	19.2	34.4	11.5	46.4	7.5

SERIES VI.—*Nine nuts from same tree as Series V, but fairly ripe.*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.				Copra (anhydrous).	
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.	Weight.	Per cent oil.
1[a]	1,644	602	36.6	1,042	63.4	207	12.6	460	28.0	50.7	49.3	233	63.8
2[a]	1,670	650	38.9	1,020	61.1	201	12.0	455	27.3	54.6	45.4	248	62.7
3[b]	2,300	1,115	48.4	1,185	51.6	229	10.0	506	22.0	51.8	48.2	262	64.3
4[b]	2,164	1,075	49.7	1,089	50.3	217	10.0	465	21.4	50.5	49.5	235	64.1
5[c]	2,519	1,294	51.4	1,225	48.6	231	9.1	501	19.9	49.5	50.5	248	64.1
6[c]	1,948	992	50.9	956	49.1	186	9.6	400	20.5	53.6	46.4	215	65.8
7[c]	3,467	2,262	65.2	1,205	34.8	232	6.7	473	13.7	45.5	54.5	215	66.9
8[c]	2,512	1,440	57.3	1,072	42.7	197	7.9	450	17.9	50.4	49.6	227	64.9
9[c]	3,230	1,985	61.4	1,245	38.6	229	7.1	471	14.6	44.3	55.7	208	65.7
Average	2,384	1,268	51.1	1,116	48.9	214	9.4	405	20.6	50.1	49.9	232	64.7

No.	Copra (anhydrous).		Milk.				Oil.		Calculated to per cent in nut free from husk.				
	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Per cent solids.	Per cent water.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	36.2	14.2	375	22.8	4.4	[d]95.6	149	9.0	19.9	44.1	22.4	36.0	14.3
2	37.3	14.9	364	21.8	4.1	[d]95.9	156	9.3	19.7	44.6	24.3	35.7	15.3
3	35.7	11.4	450	19.6	5.0	95.0	169	7.3	19.3	42.7	22.1	38.0	14.2
4	35.9	10.8	407	18.8	4.9	95.1	151	7.0	19.9	42.7	21.5	37.4	13.8
5	35.9	9.8	493	19.6	5.7	94.3	159	6.3	18.9	40.9	20.3	40.2	13.0
6	34.2	11.0	370	19.0	5.2	94.8	142	7.3	19.5	41.8	22.5	38.7	14.8
7	33.1	6.2	500	14.4	5.6	94.4	144	4.1	19.3	39.2	17.8	41.5	11.9
8	35.1	9.0	425	16.9	5.8	94.2	147	5.9	18.4	42.0	21.2	39.6	13.7
9	34.3	6.4	545	16.9	6.0	94.0	137	4.2	18.4	37.8	16.7	43.8	11.0
Average	35.3	10.4	437	18.9	5.2	94.8	150	6.7	19.3	41.7	21.0	39.0	13.6

[a] "Dead ripe." [b] "Ripe." [c] "Fairly ripe." [d] Oil separated.

Series VII.—*Nuts from same tree as Series V, but dead ripe.*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.				Copra (anhydrous).	
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.	Weight.	Per cent oil.
1	1,624	545	33.6	1,079	66.4	217	13.4	462	28.4	51.9	48.1	240	63.9
2	1,493	486	32.6	1,007	67.4	202	13.5	450	30.1	55.1	44.9	248	66.0
3	1,427	547	38.3	880	61.7	185	13.0	415	29.1	58.6	41.4	243	66.6
4	1,495	445	29.8	1,050	70.2	187	12.5	438	29.3	45.3	54.7	198	65.3
5	1,568	528	33.7	1,040	66.3	202	12.9	441	28.1	53.5	46.5	236	64.2
6	1,437	472	32.8	965	67.2	198	13.8	437	30.4	56.7	43.3	248	67.1
7	1,716	631	36.8	1,085	63.2	202	11.8	476	27.7	51.8	48.2	246	67.5
8	1,564	489	31.3	1,075	68.7	206	13.1	483	30.9	53.3	46.7	261	65.0
9	1,452	450	31.0	1,002	69.0	188	13.0	436	30.0	53.5	46.5	233	65.6
10	1,806	612	33.9	1,194	66.1	219	12.1	496	27.5	53.6	46.4	266	68.3
Average	1,558	520	33.4	1,038	66.6	201	12.9	453	29.1	53.3	46.7	242	66.0

No.	Copra (anhydrous).		Milk.				Oil.		Calculated to per cent in nut free from husk.				
	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Per cent solids.	Per cent water.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	36.1	14.8	400	24.6	4.5	95.5	153	9.4	20.1	42.8	22.2	37.1	14.2
2	34.0	16.6	355	23.8	4.3	95.7	164	11.0	20.0	44.7	24.7	35.3	16.3
3	33.4	17.1	280	19.6	5.0	95.0	162	11.3	21.0	47.2	27.6	31.8	18.4
4	34.7	13.3	425	28.4	4.3	95.7	129	8.6	17.8	41.7	18.9	40.5	12.3
5	35.8	15.1	397	25.3	4.1	95.9	152	9.7	19.4	42.4	22.7	38.2	14.6
6	32.9	17.2	330	23.0	4.5	95.5	166	11.6	20.5	45.3	25.7	34.2	17.8
7	32.5	14.4	407	23.7	4.0	96.0	166	9.7	18.6	43.9	22.7	37.5	15.3
8	35.0	16.7	386	24.7	4.3	95.7	170	10.9	19.2	44.9	24.3	35.9	15.8
9	34.4	16.1	378	26.0	4.6	95.4	158	10.5	18.8	43.5	23.3	37.7	15.3
10	31.7	14.7	479	26.5	4.3	95.7	182	10.1	18.3	41.6	22.3	40.1	15.2
Average	34.0	15.6	384	24.6	4.4	95.6	160	10.3	19.4	43.8	23.4	36.8	15.5

While there is still some individual variation among nuts from the same tree, these last analyses very conclusively show the change which is taking place as the fruit becomes riper. The average percentages of copra and oil, for example, in the nut free from husk in the green fruit, are only 11.5 and 7.5, respectively, but they rise to 21 and 13.6 in the "fairly ripe" nuts, and assume a maximum of 23.4 and 15.5 in the case of the series which had been allowed completely to ripen while still on the tree. This gain is partially due to an increase in the percentage of meat, which runs 34.4, 41.7, and 43.6 in Series V, VI, and VII, respectively, at the expense of milk, which falls from 46.4 to 39 and finally to 36.8, but it is also largely accounted for by the increase of solid matter and loss of water in the former. The percentage of anhydrous copra in

the meat of the green fruit is 33.7; it rises to 50.1 in that of the "fairly ripe" nuts and increases to 53.3 in those marked "dead ripe." The "fairly ripe" nuts which had been sent to Manila showed 51.4 per cent of anhydrous copra in the meat after standing during one month, and, after two, 53.9 per cent, this last figure being very nearly the same as that obtained from the "dead-ripe" nuts taken directly from the tree. The amount of oil obtainable from this copra also seems slightly to increase with age, running 64.5, 64.7, and 66 in the three series (V, VI, and VII), and in those nuts which had stood for one and two months it was found to be 67.09 and 67.11, respectively. However, it is also quite possible that these changes of oil content in the copra in greater part are due to individual variation in the nuts themselves.

Another interesting fact brought out by these analyses is the gradual decrease of the amount of the total solids in the milk as a nut grows riper. In green nuts this quantity averaged 6.3 per cent and the milk has a sweet, pleasant taste and is saturated with a gas which I have proven to be carbon dioxide. The occurrence of an alcoholic fermentation in the center of a sound, growing fruit, with absolutely no access of air to the milk inside, is practically impossible, and, besides, analytical tests have proven the absence of alcohol in the fresh milk, so that probably the carbon dioxide is a by-product of a process, possibly due to enzymes, which is constantly changing sugar and water into fat and cellulose. The milk from the nuts called "fairly ripe" was not so pleasant to the taste, contained very little, if any, carbon dioxide, and had decreased in total solids to 5.2 per cent; while the "dead-ripe" samples produced a milk which was rather insipid, which contained no gas, and which in most cases had a few drops of clear oil floating on the surface; the total solids in the latter had been further reduced to 4.4 per cent.

Changes taking place during the ripening of a coconut.—From the foregoing data, and from observations made on very young nuts, the following are probably the changes which a young coconut undergoes before it reaches maturity:

When the young fruit first appears it consists of a white, astringent tasting, semifibrous mass, which afterwards is destined to form the husk; and of a thin, green outer skin. The nut gradually increases in size, with very little change in composition, until it has grown to be about 3 inches in diameter. It then has a comparatively small, hollow space in the center which is completely filled with a watery fluid of an astringent, slightly acid taste, and which is much like the juice from a green husk. As this period begins, a rudimentary shell is formed around the inner surface of the nut; at first this is very thin and soft, but slowly it becomes thicker and harder. Not until the nut has reached its maximum size, with its shell completed, is there any indication of meat or of oily material. When the shell has been formed the milk changes in character,

it becomes rather sweet, and a slimy, gelatinous mass, having a sweetish taste and containing comparatively little oil begins to deposit on the inside of the former. At first this forms chiefly on the lower half of the nut, but finally it covers the whole inner surface. This pulpy mass soon grows thicker and denser, it increases in oil content at the expense of sugar in the milk, until it assumes the well-known characteristics of ordinary coconut meat. During this last stage the evolution of carbon dioxide which previously was mentioned occurs. Even in ripe nuts, after they have been picked from the tree, there seems to be a slight continuation of the hardening process in the meat, covering a period of from two to three months, or until the sprout makes its appearance. Then other changes occur, the reverse of those which had taken place previously; the nourishment concentrated and stored up as fat is now transformed into sugars and other bodies capable of being directly assimilated by the young plant. As this process goes on the embryo or "foot" gradually increases in size until it occupies the whole space inside the nut and makes use of all the nourishment contained therein for the growth of the young tree.

Therefore, for the largest yield of copra and oil, only thoroughly ripe nuts (the husks of which have begun to turn brown) should be used, and it is often advisable to allow the latter to stand in a dry place for a few weeks before they are opened. The greatest care should be taken to avoid using green nuts, as it is shown by the tables given above that a loss of almost 50 per cent may thus result.

On the other hand, coconuts should not be stored too long, for in about three months the embryo begins to grow, and, even before that time, those nuts which may have been cracked or bruised in gathering, have a tendency to become rancid.

Analysis of nuts of different color.—In a certain portion of San Ramon farm there exist, growing side by side in the same kind of soil, two apparently different varieties of coconut trees, one of which uniformly produces nuts of a golden-yellow color, while the other bears a light-green fruit. Both varieties eventually turn brown at maturity. Analyses of these nuts are given in the accompanying tables, Series VIII being ten ripe nuts from a tree which bears a green fruit, while Series IX is made up of nuts from a tree about 50 feet away whose product is yellow until it becomes "dead ripe."

SERIES VIII.—*Ten thoroughly ripe nuts from one tree.*

[The nuts on this tree all have a green husk until they become "dead ripe," when they change to a dull brown.]

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.			
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1	1,490	585	39.3	905	60.7	204	13.7	397	26.6	44.6	55.4
2	2,160	640	29.6	1,520	70.4	275	12.4	663	30.7	52.6	47.4
3	1,632	657	40.3	975	59.7	227	13.9	428	26.2	46.3	53.7
4	1,482	437	29.5	1,045	70.5	235	15.9	438	29.5	44.4	55.6
5	1,862	665	35.7	1,197	64.3	265	14.2	482	25.9	44.4	55.7
6	1,517	395	26.0	1,122	74.0	255	16.8	467	30.8	53.5	46.5
7	1,702	567	33.3	1,135	66.7	260	15.3	498	29.2	46.8	58.2
8	1,623	520	32.0	1,103	68.0	265	16.4	476	29.3	51.3	48.6
9	1,900	700	36.8	1,200	63.2	270	14.2	505	26.6	44.6	55.3
10	1,673	475	28.4	1,198	71.6	210	12.6	521	31.1	57.0	43.0
Average	1,704	564	33.1	1,140	66.9	247	14.6	488	28.6	48.5	51.5

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	177	64.2	35.8	11.9	304	20.4	114	7.6	22.5	43.9	19.6	33.6	12.6
2	349	62.5	37.5	16.1	582	26.9	218	10.1	18.1	43.6	23.0	38.3	14.3
3	198	62.7	37.3	12.1	320	19.6	124	7.6	23.3	43.9	16.1	32.8	12.7
4	195	62.6	37.4	13.1	372	25.1	122	8.2	22.5	41.9	18.7	35.6	11.7
5	214	60.8	39.2	11.5	450	24.2	130	7.0	22.1	40.3	17.9	37.6	10.9
6	250	65.0	35.0	16.5	380	25.7	163	10.7	22.7	41.6	22.3	34.8	14.5
7	233	67.0	33.0	13.7	377	22.2	170	9.2	22.9	43.9	20.5	33.2	13.8
8	242	64.3	35.7	14.9	362	22.3	156	9.6	24.0	43.2	22.0	32.8	14.1
9	225	65.9	34.1	11.9	425	22.4	148	7.8	22.5	42.1	18.8	35.4	12.4
10	297	64.6	35.4	17.7	467	27.9	192	11.5	17.5	43.5	24.8	39.0	16.0
Average	238	64.0	36.0	13.9	405	23.7	154	8.9	21.8	42.8	20.4	35.3	13.3

SERIES IX.—*Ten thoroughly ripe nuts from one tree.*

[These nuts have a golden-yellow color until dead ripe, when they look like those of Series VIII.]

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.			
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1	1,613	428	26.5	1,185	73.5	230	14.3	495	30.7	47.1	52.9
2	1,960	520	26.7	1,440	73.3	256	13.0	589	30.0	53.6	46.4
3	1,657	375	22.6	1,282	77.3	240	14.5	547	33.0	53.4	46.6
4	1,608	353	22.0	1,255	78.0	235	14.5	545	33.9	52.7	49.3
5	1,653	335	20.3	1,318	79.7	245	14.8	568	34.4	53.1	46.9
6	1,577	560	35.5	1,017	64.5	205	13.0	432	27.4	49.6	51.3
7	1,780	510	28.7	1,270	71.3	252	14.2	533	29.9	53.6	46.4
8	1,650	380	23.0	1,270	77.0	247	15.0	538	32.6	51.2	48.8
9	1,597	497	31.1	1,100	68.9	225	14.2	495	30.9	43.4	56.6
10	1,926	486	25.2	1,440	74.8	257	13.4	591	30.7	51.2	48.8
Average	1,702	444	26.2	1,258	73.8	239	14.1	533	31.3	50.9	49.1

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	233	Copra destroyed en route; assume same per cent as in Series VIII: 64.		14.5	460	28.5	149	9.2	19.4	41.8	19.6	38.8	12.6
2	315			16.1	595	30.3	202	10.3	17.8	40.9	21.9	41.3	14.0
3	292			17.6	495	29.8	187	11.3	18.7	42.7	22.8	38.6	14.6
4	287			17.8	475	29.6	184	11.4	18.7	43.4	22.9	37.9	14.6
5	301			18.2	505	30.5	193	11.7	18.6	43.1	22.8	38.3	14.6
6	214			13.6	380	24.1	137	8.7	20.1	42.5	21.1	37.4	13.5
7	285			16.0	485	27.2	182	10.2	19.9	42.0	22.4	38.2	14.4
8	276			16.7	485	29.4	177	10.7	19.5	42.9	21.7	38.2	13.9
9	215			13.5	380	23.8	138	8.6	20.5	45.0	19.5	34.5	12.5
10	302			15.7	592	30.7	193	10.0	17.9	41.0	21.0	41.1	13.4
Average	272	-------	---	16.0	485	28.4	174	10.2	19.1	42.5	21.6	38.4	13.8

Very little difference can be observed between these two varieties; the average weight is almost exactly the same; the percentage of husk and shell is somewhat lower in the yellow nuts, but this advantage to a large extent is counterbalanced by their percentage in milk, so that the amount of meat in the two remains practically the same. The yellow nuts average 272 grams of anhydrous copra against 238 grams in the green ones, which is quite decidedly in favor of the former.

Unfortunately, the copra from Series IX was spoiled in transit to Manila. Calculations on the oil contents of this series were therefore based on the assumption that this copra would have contained 64 per cent oil—that is, the same percentage as that found in Series VIII. Figuring the yield of oil on this basis, we have an average of 174 grams for the yellow nuts against 154 for the green ones. However, it will be noticed that these tables show a difference of over 100 grams in each series between the maximum and minimum weight of oil, therefore if another series of analyses of nuts from these two trees were to be made possibly the slight advantage in favor of the yellow nuts might be reversed. At any rate it may be concluded that the color of a nut has very little, if any, influence on its composition.

Nuts from different localities.—In order to test the truth of the statement that coconuts produced by trees growing along the seashore are of a quality superior to those taken from farther inland, ten nuts were selected at random from a large pile gathered near the sea and analyzed as shown in the accompanying Series X, while a like number was secured from a similar one containing the product of trees growing some 1,800 feet inland (Series XI).

Series X.—*Ten nuts from a pile of 1,000 taken from trees near the sea.*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.			
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1[a]	3,125	1,420	45.4	1,705	54.6	310	9.9	745	23.8	54.3	45.7
2[b]	2,165	638	29.2	1,532	70.8	285	13.2	627	29.0	59.1	40.9
3[c]	2,520	1,210	48.0	1,310	52.0	301	12.0	549	21.8	54.8	45.2
4[b]	3,492	1,560	44.7	1,932	55.3	425	12.1	775	22.2	45.0	55.0
5[a]	2,292	992	43.3	1,300	56.7	300	13.1	608	26.5	57.8	42.2
6[d]	3,215	1,355	42.1	1,860	57.9	359	11.2	701	21.8	49.8	50.2
7[d]	2,785	1,405	50.4	1,380	49.6	291	10.5	594	21.3	42.2	57.8
8[b]	2,512	792	31.5	1,720	68.5	340	13.5	738	29.4	57.3	42.7
9[e]	3,240	1,320	40.8	1,920	59.2	380	11.7	780	24.1	51.5	48.5
10[d]	2,765	1,170	42.3	1,595	57.7	262	9.5	683	24.7	49.7	50.3
Average	2,811	1,186	41.8	1,625	58.2	325	11.7	680	24.4	52.2	47.8

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	404	65.3	34.7	12.9	650	20.9	264	8.4	18.2	43.7	23.7	38.1	15.5
2	370	67.3	32.7	17.1	620	28.6	249	11.5	18.6	40.9	24.2	40.5	16.3
3	306	65.7	34.3	12.1	460	18.2	201	8.0	23.0	41.9	23.4	35.1	15.4
4	349	64.0	36.0	10.0	732	21.0	224	6.4	22.0	40.1	18.1	37.9	11.6
5	349	69.8	30.2	15.2	392	17.1	244	10.1	23.1	46.8	26.8	30.1	18.7
6	349	......	......	10.9	800	24.9	......	......	19.3	37.7	18.8	43.0	......
7	251	61.6	38.4	9.0	495	17.8	155	5.6	21.1	43.0	18.2	35.9	11.2
8	423	63.6	36.4	16.8	612	25.6	269	10.7	19.8	42.9	24.6	37.3	15.6
9	402	62.8	37.2	12.4	760	23.4	253	9.5	19.8	40.6	20.9	39.6	13.2
10	339	......	......	12.3	650	23.5	......	......	16.4	42.8	21.3	40.8	......
Average	354	65.0	35.0	12.9	620	22.1	232	8.8	20.1	42.1	22.0	37.8	14.7

[a] Yellow-green. [b] Brown. [c] Yellow. [d] Green. [e] Brown-yellow.

SERIES XI.—*Ten nuts from a pile of 1,000 taken from trees inland about 1,800 feet.*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.			
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.
1[a]	4,114	1,612	39.2	2,502	60.8	540	13.1	857	20.8	45.3	54.7
2[b]	2,500	865	34.6	1,635	65.4	352	14.1	726	29.0	50.9	49.1
3[b]	2,512	652	26.0	1,860	74.0	352	14.0	746	29.7	57.0	43.0
4[b]	2,763	863	31.2	1,900	68.8	337	12.2	766	27.7	45.4	54.6
5[b]	2,745	815	29.7	1,930	70.3	343	12.5	752	27.4	51.2	48.8
6[c]	4,102	1,592	38.8	2,510	61.2	452	11.0	911	22.2	45.5	54.5
7[b]	2,485	525	21.1	1,960	78.9	345	13.9	820	33.0	49.3	50.7
8[b]	1,423	425	29.9	998	70.1	235	16.5	473	33.2	59.3	40.7
9[a]	2,675	1,000	37.4	1,675	62.6	342	12.8	603	22.5	37.3	62.7
10[d]	3,620	1,170	32.3	2,450	67.7	489	12.1	936	25.9	49.3	50.7
Average	2,894	952	32.0	1,942	68.0	374	13.2	759	27.2	49.1	50.9

No.	Copra (anhydrous).				Milk.		Oil.		Calculated to per cent in nut free from husk.				
	Weight.	Per cent oil.	Per cent pulp.	Per cent copra in nut.	Weight.	Per cent.	Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	388	57.7	42.3	9.4	1,105	26.9	224	5.4	21.6	34.2	15.5	44.2	9.0
2	369	59.8	40.2	14.8	557	22.3	221	8.8	21.5	44.4	22.6	34.1	13.5
3	425	65.1	34.9	16.9	762	30.3	277	11.0	18.9	40.1	22.8	41.0	14.9
4	348	59.3	40.7	12.6	797	28.9	206	7.5	17.7	40.3	18.3	42.0	10.9
5	385	63.7	36.3	14.0	835	30.4	245	8.9	17.8	38.9	20.0	43.3	12.7
6	415	62.0	38.0	10.1	1,147	28.0	257	6.3	18.0	36.3	16.5	45.7	10.3
7	404	58.8	41.2	16.3	795	32.0	238	9.6	17.6	41.8	20.6	40.6	12.1
8	280	61.4	38.6	19.7	290	20.4	172	12.1	23.5	47.4	28.1	29.1	17.2
9	225	63.5	36.5	8.4	730	27.3	143	5.3	20.4	36.0	13.4	43.6	8.5
10	461	67.8	32.2	12.7	1,075	29.7	313	8.6	17.9	38.2	18.8	43.9	10.6
Average	370	61.9	38.1	13.5	809	27.6	230	8.4	19.5	39.8	19.7	40.7	12.0

[a] Green. [b] Brown. [c] Yellow-green. [d] Yellow.

In selecting nuts for the two preceding series of analyses no attempt was made to secure uniformity as to size and age. On the contrary, they were picked out with a view of obtaining fairly representative samples of the largest and of the smallest, as well as of the most and of the least mature in each pile, so that they would vary through a wide range of color and weight. On comparing the two lots it will be seen that the results agree very closely. Series XI averages a little higher in the total yield of copra, but the oil content of this copra is somewhat lower than in Series X, so that they yield almost exactly the same quantity of oil per nut. The proportion of husk taken from the seashore nuts (41.8) is much larger than it is from those gathered from the interior (32), but

this is compensated for by the fact that the percentage of milk in the nut, free from husk, and of water in the fresh meat is considerably lower in the former than in the latter. Therefore it appears to be very evident that the superiority of trees growing near the sea is solely due to the quantity and not to the quality of nuts they produce.

Analyses of large numbers of nuts.—As a check on these last results, secured on a small scale, it was decided to determine the actual weight of the various products of the coconut under the conditions ordinarily obtaining in the manufacture of commercial copra, and, with this end in view, 1,000 nuts were procured from trees growing near the seashore and the same number from those standing in the interior. After lying for one month the nuts were put through the regular process for making copra which has previously been described. The weight in pounds of the whole nuts, husks, meat and shells, dried shells, and copra was determined directly on an ordinary Fairbanks scale, the meat and milk being obtained by difference. Five hundred nuts from each lot were sun dried and 500 grill dried and the resulting weight of copra multiplied by two to give the yield of 1,000 nuts by each method. For the determination of moisture and oil in this copra, twenty samples were taken from each lot, cut into small pieces, and quartered down to about 100 grams. The moisture was determined at once, after which the copra was sealed and sent to Manila to secure the determination of the oil content. Both moisture and oil were determined in triplicate.

SERIES XII.

Portion determined.	Seashore nuts.		Inland nuts.	
	Weight in kilos.	Per cent.	Weight in kilos.	Per cent.
Weight of 1,000	2,363	100.0	2,286	100.0
Husks	897	38.0	703	30.8
Nuts minus husks	1,466	62.0	1,582	69.2
Meat and shell	929		979	
Milk	537	22.7	603	26.4
Shell (dry)	282	11.9	291	12.7
Meat	647	27.4	688	30.1

Portion determined.	Seashore nuts.				Inland nuts.			
	Sun dried.		Grill dried.		Sun dried.		Grill dried.	
	Weight in kilos.	Per cent.	Weight in kilos.	Per cent.	Weight in kilos.	Per cent.	Weight in kilos.	Per cent.
Copra	302.1	12.8	330.2	14.0	322.9	14.1	333.0	14.6
Oil	182.2	7.7	198.9	8.4	191.1	8.4	189.8	8.3
Moisture in copra		9.2		8.6		9.8		10.1
Oil in copra		60.3		60.2		59.2		57.0

This work, performed as it was on a large scale, agrees rather more closely with the results obtained from the series of ten nuts each than was to be expected. Here, again, it may be observed that the proportion of husk from the seashore nuts is considerably higher than it is from those from the interior, while the total amount of water is correspondingly less, so that nuts from the two localities yield practically the same amount of copra and oil.

While weighing out 1,000 nuts from the seashore trees it was found that 55 of them, or 5.5 per cent, were in such a bad condition as to be unfit for making copra, and fresh nuts had to be substituted. Out of the same number from the interior only 15 were spoiled. The cause of this difference is probably found in the fact that the nuts from trees near the sea fall upon harder ground and are therefore more apt to become bruised and injured, and it is very possible that the inferior yield of sun-dried as compared with kiln-dried copra, in the case of the seashore nuts, is due to this. Given perfectly sound coconuts, the two methods of drying should produce equal amounts of copra, but a green nut, or one which has begun to decay, would undoubtedly be more subject to the attacks of mold, bacteria, and insects during the comparatively long alternate heating and cooling incident to the sun-drying process than if it were dried quickly at a higher temperature.

The figures obtained in this last series on a commercial basis establish, even more firmly than do the results of analyses alone, the fact that there is practically no difference in quality between the nuts gathered along the seashore and those from farther inland. They should also be of some value as representing the average yield in copra and oil from nuts produced in the southern parts of the Islands.

NUTS FROM DAVAO.

The following analyses were made of ripe coconuts, collected near Davao, about 1 mile inland from the sea. In this region two varieties of trees have been noticed, one producing large nuts rather pointed in shape, the other bearing a smaller, rounder fruit.

Series XIII consists of ten of the small nuts, Series XIV of the large variety. On examining these figures it will be noticed that Series XIII shows very much the same proportion of its various constituents, as well as the total of oil, as the average lot of ripe nuts from San Ramon.

Series XIV excels in total weight of oil simply because it is made up of larger nuts. The percentage of oil in the nut, free from husk, is the same in both series. The nuts in these two series were fairly uniform in composition, with the exception of No. 7 in Series XIV, which had a total weight of only 92 grams of oil, less than one-half of the average amount.

SERIES XIII.—*Davao nuts, small.*

No.	Total weight.	Husk. Weight.	Husk. Per cent.	Nut minus husk. Weight.	Nut minus husk. Per cent.	Shell. Weight.	Shell. Per cent.	Meat. Weight.	Meat. Per cent.	Meat. Per cent copra.	Meat. Per cent water.
1	2,016	480	23.8	1,536	76.2	296	14.7	672	33.3	48.2	51.8
2	2,220	548	24.7	1,672	75.3	347	15.6	639	28.8	53.5	46.5
3	1,902	385	20.2	1,517	79.8	317	16.7	644	33.9	55.8	44.2
4	2,151	404	18.8	1,747	81.2	356	16.5	721	33.5	55.0	45.0
5	2,153	488	22.7	1,665	77.3	360	16.7	665	30.9	51.5	48.5
6	1,823	455	25.0	1,368	75.0	272	14.9	531	29.1	43.8	56.2
7	2,232	427	19.1	1,805	80.9	337	15.1	786	35.2	42.8	57.2
8	2,019	451	22.3	1,568	77.7	300	14.9	648	32.1	48.3	51.7
9	2,157	396	18.3	1,761	81.7	370	17.2	673	31.2	50.6	49.4
10	2,098	364	17.3	1,734	82.7	382	18.2	717	34.2	49.2	50.8
Average	2,077	440	21.2	1,637	78.8	334	16.1	669	32.2	49.9	50.1

No.	Copra. Weight.	Copra. Per cent copra in nut.	Copra. Per cent oil.	Copra. Per cent pulp.	Milk. Weight.	Milk. Per cent.	Oil. Weight.	Oil. Per cent.	Calculated to per cent in nut free from husk. Shell.	Meat.	Copra.	Milk.	Oil.
1	324	16.1	61.1	38.9	568	28.2	198	9.8	19.3	43.7	21.1	37.0	12.9
2	342	15.4	40.6	59.4	686	30.9	139	6.3	20.8	38.2	20.5	41.0	8.3
3	360	18.9	51.5	48.5	556	29.2	185	9.7	20.9	42.5	23.7	36.6	12.2
4	396	18.4	58.6	41.4	670	31.2	232	10.8	20.4	41.3	22.7	38.3	13.3
5	343	15.9	58.2	41.8	640	29.7	200	9.3	21.6	39.9	20.6	38.5	12.0
6	233	12.8	60.8	39.2	565	31.0	142	7.8	19.9	38.8	17.0	41.3	10.4
7	336	15.1	58.6	41.4	682	30.6	197	8.8	18.7	43.5	18.6	37.8	10.9
8	313	15.5	54.5	45.5	620	30.7	171	8.4	19.1	41.3	20.0	39.6	10.9
9	340	15.8	52.3	47.7	718	33.3	178	8.2	21.0	38.2	19.3	40.8	10.1
10	353	16.8	51.2	48.8	635	30.3	181	8.6	22.0	41.4	20.4	36.6	10.4
Average	334	16.1	54.7	45.3	634	30.5	182	8.8	20.4	40.9	20.4	38.7	11.1

SERIES XIV.—*Davao nuts, large.*

No.	Total weight.	Husk.		Nut minus husk.		Shell.		Meat.				Copra.	
		Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Weight.	Per cent.	Per cent copra.	Per cent water.	Weight.	Per cent copra in nut.
1	3,070	910	29.6	2,160	70.4	452	14.7	766	25.0	43.7	56.3	335	10.9
2	3,092	725	23.4	2,367	76.6	482	15.6	846	27.4	52.3	47.7	442	14.3
3	2,391	614	25.7	1,777	74.3	308	12.9	763	31.9	53.9	46.1	411	17.2
4	2,737	639	23.4	2,098	76.6	340	12.4	861	31.5	47.7	52.3	411	15.0
5	2,483	752	30.3	1,731	69.7	333	13.4	715	28.8	54.5	45.5	390	15.7
6	2,367	765	32.3	1,602	67.7	320	13.5	686	29.0	43.9	56.1	301	12.7
7	1,897	608	32.0	1,289	68.0	244	12.9	585	28.2	31.2	68.8	167	8.8
8	2,271	663	29.2	1,608	70.8	327	14.4	688	30.3	47.4	52.6	326	14.4
9	2,444	495	20.2	1,949	79.8	330	13.5	794	32.5	49.9	50.1	396	16.2
10	2,526	516	20.4	2,010	79.6	323	12.8	799	31.6	51.6	48.4	413	16.3
Average	2,528	669	26.7	1,859	73.3	346	13.6	745	29.6	47.6	52.4	360	14.2

No.	Copra.		Milk.		Sprout.	Oil.		Calculated to per cent in nut free from husk.				
	Per cent oil.	Per cent pulp.	Weight.	Per cent.		Weight.	Per cent.	Shell.	Meat.	Copra.	Milk.	Oil.
1	59.3	40.7	942	30.7		199	6.5	20.9	35.5	15.5	43.6	9.2
2	62.9	37.1	1,039	33.6		278	9.0	20.4	35.7	18.7	43.9	11.7
3	60.7	39.3	700	29.3	6	250	10.4	17.3	42.9	23.1	39.4	14.0
4*	64.1	35.9	892	32.6	5	263	9.6	16.2	41.1	19.6	42.5	12.6
5*	65.7	34.3	680	27.4	3	256	10.3	19.2	41.3	22.5	39.3	14.8
6*	64.9	35.1	596	25.2		195	8.3	20.0	42.8	18.8	37.2	12.2
7*	55.3	44.7	504	26.5	6	92	4.9	18.9	41.5	13.0	39.1	7.2
8*	61.0	38.0	589	25.9	4	199	8.8	20.3	42.8	20.3	36.6	12.4
9*	63.8	36.2	825	33.8		253	10.3	16.9	40.8	20.3	42.3	13.0
10*	60.8	39.2	875	34.6	18	251	9.9	16.1	39.8	20.6	43.5	12.2
Average	61.9	38.1	764	30.0	3	224	8.8	18.6	40.4	19.2	40.7	12.0

*Oil separated from the milk, hence the nuts were very ripe.

[*To be followed by a paper on "The Keeping Qualities and the Causes of Rancidity in Coconut Oil" in the next number of the* JOURNAL.]

PLATE I. COCONUT PALMS GROWING ON THE BEACH AT SAN RAMON, SHOWING HABITAT.

PLATE II. THE NUTS SET OUT IN THE SEEDING BEDS.

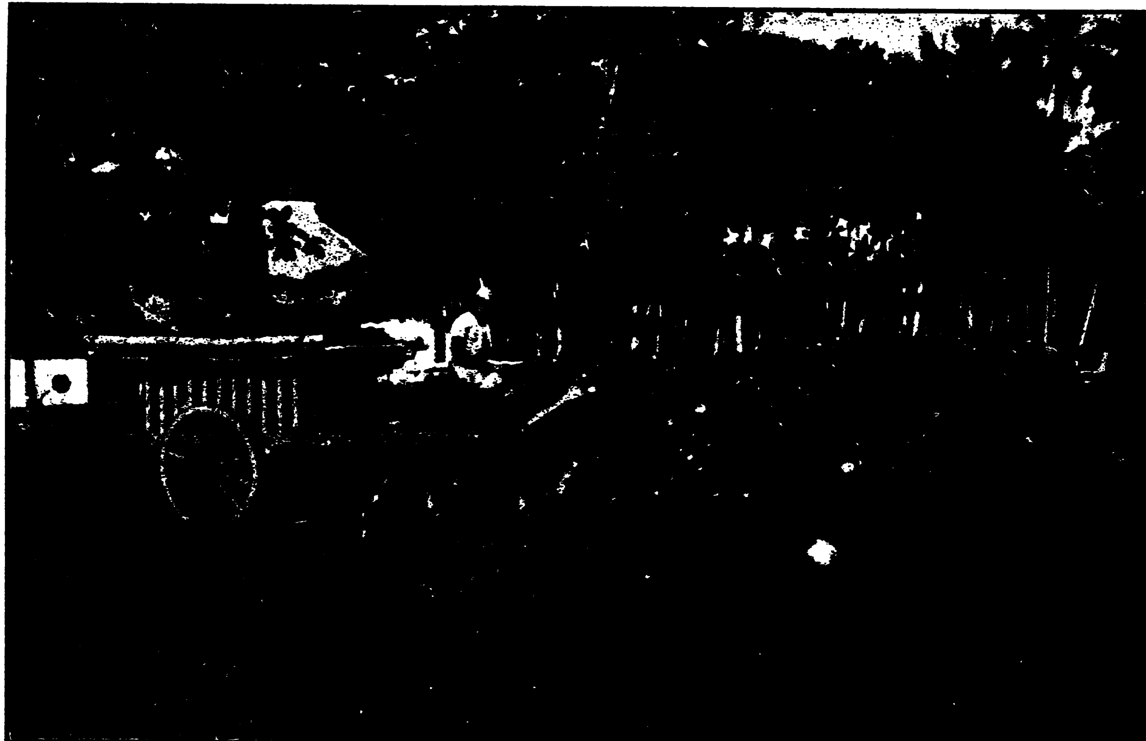

PLATE III. GATHERING COCONUTS IN PILES NEAR THE DRYING SHEDS.

PLATE IV. SORTING AND HUSKING NUTS ON THE BEACH NEAR THE DRYING SHEDS.

PLATE V. METHOD OF HUSKING THE COCONUT.

PLATE VI. BREAKING OPEN THE COCONUT BEFORE DRYING; THE MILK GOES TO WASTE ON THE GROUND.

PLATE VII. SUN DRYING THE NUTS ON TRAYS.

PLATE VIII. SUN DRYING, SHOWING THE NUTS ON THE TRAYS, READY TO BE PUSHED UNDER THE SHELTER.

PLATE IX. KILN USED FOR DRYING NUTS.

PLATE X. KILN DRYING; THE HALVES OF THE COCONUTS ARE PLACED OVER THE GRILL FOR THE PRELIMINARY DRYING.

THE OCCURRENCE OF SCHISTOSOMA JAPONICUM VEL CATTOI IN THE PHILIPPINE ISLANDS.

By Paul G. Woolley.
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As long ago as 1887 Mazima, in Japan, wrote of a peculiar form of liver cirrhosis which was caused by an unknown parasite. In succeeding years his observations received corroboration from various sources. The ova of this parasite were found not only in the liver but also in other organs, and it soon became apparent that the observers were dealing with a definite endemic disease which was more or less closely confined to the Provinces of Bingo, Yamanashi, Hiroshima, and Saga. From a town in Bingo (Katayama) the malady has taken its name, so that in Japan it is known as the "Katayama disease."

In 1904 Katsurada studied fifteen cases of the infection, and in the stools of five found ova which resembled those of *Schistosomum hæmatobium*. Later, in dissecting dogs and cats from an infected district, he encountered (in a cat) flukes within the portal vessels. These he described (August 30, 1904) in a Japanese paper, in which he proposed the name *Schistosomum japonicum* for the parasite. Later, in December, 1904, Katsurada published again on this subject, this time in German, and stated that Fujinami had announced (October, 1904) the discovery of a female *S. japonicum* in a human subject. In the same year, in lesions of the liver, mesenteric glands, and intestines of a Chinaman from the Province of Fukien, China, Catto, at that time resident medical officer of the Singapore quarantine station, found certain bodies which he believed to be coccidia. The case was first reported as one of coccidiosis, but later this diagnosis was changed, and in September, 1904, the claim was set forth that the bodies were the ova of a new parasite. Later still, Blanchard, after seeing Catto's specimens, gave the trematode the name of *Schistosoma cattoi*, and in 1905 Catto described it under that title. Catto based his description upon material obtained from the human subject, while Katsurada based his largely upon that obtained from cats, and this distinction, as Stiles insists, must be taken into consideration.

This being the case, the conclusion is fairly safe that the parasites described from Japan and China are of the same species. It also seems

assured that they are quite different from the Egyptian form *S. hæmatobium*.

Here it is only necessary to say that the worms are characterized by the absence of the ciliated warts on the integument, which are a marked feature of *S. hæmatobium*. Minor anatomic differences are the size of the worm [average 10.43 millimeters (Katsurada)], the length of the vas deferens, and the lobular character of the testes.

The eggs are smaller than those of *S. hæmatobium*, have blunter ends, and no spine.

A complete comparison of the Chinese and Japanese worms and of their ova will be found in Stiles's paper.

The description of the clinical symptoms of the disease "Katayama" must, for the present, be taken from the Japanese reports, since in neither Catto's nor my case was there any opportunity for clinical study.

Katsurada was able to examine from 30 to 54 cases every year while stationed in the infected district, in which his residence extended over about five years. He observed but few deaths (three to five annually) which he considered were directly due to the parasite, but he regards the indirect mortality as much higher. Defective physical development is the rule in affected children. Diarrhœa is usually the first symptom to be noted, while anæmia and ascites generally follow later; however, the most striking feature is the shape assumed by the trunk. The hypogastric region seems to shrink, while the epigastric enlarges, a transverse furrow forming directly above the umbilicus, so that the general appearance of the abdominal region is that of an inverted gourd. Dilatation of the epigastric region and of the lower part of the thorax was noted even in patients whose liver and spleen were not much enlarged. The commonest symptoms are an initial increase in the size of the liver, followed by a decrease, a secondary enlargement of the spleen, a muco-sanguinous diarrhœa, severe attacks of ascites, and progressive anæmia. Katsurada found the ova of the parasite under discussion and also those of *Tricocephalus dispar*, *Uncinaria*, and *Ascaris lumbricoides* in the stools of his patients.

Yamagiwa described (1890) a case of Jacksonian epilepsy in which he found ova in certain nodules in the brain. These ova were similar to those now known to occur in "Katayama." At the time Yamagiwa first reported his case he considered these ova to be those of the lung distome, but he now believes himself to have been dealing with *Schistosoma japonicum*.

In Catto's case the right lobe of the liver extended for a distance of two fingers' width below the costal margin and the left lobe a hand's breadth below the sternum. The spleen was enlarged.

My case occurred in a native Filipino who had not been out of the Islands and who at the time of his death was in Bilibid Prison. He died suddenly of a terminal bacterial infection in the course of intestinal amœbiasis and uncinariasis. The liver was not enlarged, but the spleen was somewhat increased in size.

The pathologic details of the Japanese cases, as described by Katsurada (Scheube), are as follows:

At autopsy the liver is less than normal in size and its surface is marked by small nodules, larger than those observed in Laennec's cirrhosis and smaller than those of the usual gross form. The capsule of Glisson is thickened. Microscopical observation shows connective tissue increase and round-cell infiltration in the capsule of Glisson in which the ova lie, in part in the lumen or in the walls of the portal capillaries and in part in the connective tissue. There are also fibrous nodules and tubercle-like areas which contain ova, although these are not commonly seen in the parenchyma. In addition to their location in the liver, the eggs are also found in the intestinal wall (especially that of the large intestine), in the mesentery, in the mesenteric glands, the lungs, and the brain. In the intestinal wall they especially occur in the submucosa and often are present in such numbers as to cause the mucosa over them to become bulged out or even eroded. Kanamori (Scheube) found in one case, in the rectum and sigmoid, adenomas resembling the new growths described by Kartulis in *Bilharziosis*. In the lungs and brain the eggs are encountered in tubercle-like masses, surrounded by round-cell infiltration and an increase of connective tissue.

In Catto's case the liver and spleen were both enlarged. The condition of the peritoneum suggested that repeated attacks of peritonitis had occurred. The appendices epiploicæ were thickened and in places were matted together. The recto-vescical pouch was almost obliterated. The mesenteric lymph glands were enlarged. The liver was apparently cirrhotic. The colon was thickened and its mucous membrane was swollen, hyperæmic, and friable, and presented small circular, superficial erosions and patches of necrosis. The rectum was adherent to the bladder. The mucosa of the ileum was congested and formed thickened patches. The stomach, pancreas, adrenals, kidneys, heart, and lungs showed no gross lesions. In sections of the liver, mesenteric glands, and bowel small oval bodies were found which were at first believed to be coccidia. Subsequent examination disproved this and showed them to be the ova of a trematode. Nematode embryos were found in smears from the large intestine and in the vessels of a mesenteric lymphatic gland. In sections of the meso-colon, adult trematodes were found in blood vessels, and in the uterus of one of these were oval bodies corresponding to those seen free in the tissues in other sections. The parent worms were encountered in small groups at the bifurcations of the small mesenteric vessels. Where the ova had accumulated in certain places they had provoked a small-cell infiltration which gave rise to a proliferation of fibrous tissue. In the intestine, from cæcum to anus, the ova roughly occupied two concentric layers — the one subperitoneal where they were comparatively scarce, the other submucous where they were innumerable. They were also plentiful in the mucosa, and more numerous in the necrotic areas, in which situation they were seen apparently to be in the process of extrusion.

The rectum and appendix were the parts most affected in the entire intestinal tract. Ova were found throughout the small intestine, but only in patches and in comparatively small numbers. They were plentiful in the liver, lying singly or in large or small clumps embedded in the hypertrophied fibrous tissue. They were also found in the thickened trabeculæ of many of the enlarged mesenteric glands. Ova were also encountered in the outer wall of the gall bladder, in the pancreas, liver capsule, the fibrous coat of the mesenteric vessels, mesenteric, pylorus, duodenum, jejunum, and ileum. Ova of *Trichocephalus dispar* and *Ascaris lumbricoides* were also seen in the bowel.

The case to be described was one of the series which formed the basis of a report on the pathology of intestinal amœbiasis by Dr. Musgrave

and the writer. During the investigation of the pathologic anatomy of that disease I discovered the presence of the ova which, in the opinion of Shiga, Fujinami, and Stiles, are those of *S. japonicum v. cattoi.*

The autopsy was performed by Dr. Musgrave a few minutes after the death of the patient. There was an old, discharging abscess on the right arm and another on the right side of the thorax extending into the pectoral muscles. The subcutaneous fat was well preserved and the muscles were somewhat pale. The left lung showed an intense congestion, with œdema of the lower lobe. The right was also congested and an abscess, over which the two layers of the pleura were firmly adherent, was present in the lower lobe, binding the lung to the diaphragm, ribs, and sternum. The cavity of this abscess, resembling those seen in amœbiasis, was filled with a thick pus. The abdominal cavity was free from adhesions. The walls of the intestine were somewhat thickened and the mesenteric lymphatics moderately enlarged. The spleen was enlarged and a well-marked chronic perisplenitis was present; it was adherent to the diaphragm, and its surface was wrinkled and pale. The liver showed a considerable perihepatitis and was bound to the diaphragm and abdominal wall by firm adhesions. On the dome was a large scar resembling that resulting from a healed abscess; about this were old and dense adhesions. On section, the liver was pale and cloudy, giving an increased resistance when cut. The kidneys showed a moderate parenchymatous degeneration. The stomach and the small intestine showed a well-marked catarrhal condition, and in the former there were a few small hæmorrhages. In the upper 40 centimeters of the small intestine there were a number of uncinaria. The large bowel gave evidence of amœbic infection throughout, but the most marked pathologic changes were in the transverse and descending colon, and less in the cæcum and rectum. In the most advanced lesions the process simulated a hæmorrhagic enteritis in which small superficial ulcerations predominated. These ulcerations displayed a considerable variety, but the deep-sloughing, undermined ulcer was not present. The appendix was not involved. (Musgrave.)

Microscopically, large numbers of amœbæ were found in scrapings from the ulcers and in the intestinal contents, but none could be demonstrated in the pulmonary abscess. Ova of uncinaria were also present in the intestinal contents.

Tissues from the intestine, liver, and lungs were secured and preserved in Kaiserling's solution. Bits of these were embedded in celloidin and paraffin. Sections were stained with hematoxylin and eosin.

The histological study showed that the mucous membrane of the large intestine was atrophied and, in areas, eroded. The submucosa was thickened and œdematous. The muscular layers presented but little change. The ova occurred chiefly in fibroid tissue in the submucosa, where they were innumerable and surrounded by round-celled infiltration. In the

mucosa they were much fewer, in the subperitoneal layer very infrequent, in the muscular layer absent. In the liver they were confined almost entirely to the perivascular tissues, and were most commonly seen about the intralobular vessels. They also occurred about the interlobular vessels and in the parenchyma. In the lungs they were found only in the tissue about the abscess cavity and were seen in but very small numbers. Wherever they were present they were surrounded by small-cell infiltration and fibrosis.

The following comparative measurements of the ova were furnished to me by Dr. Shiga, after he had examined my specimens and compared them with those of Fujinami and Manson:

	Manson.	Fujinami.	Woolley.
	mm.	*mm.*	*mm.*
Length	0.0728	0.0662	0.0624
Breadth	.048	.0436	.0436

In the opinion of Katsurada these parasites feed upon the blood and in this way produce the anæmia which, according to the Japanese reports, is a common symptom of the disease. He also (see Stiles) suggests that the worms probably form a toxin which perhaps is the cause of the enlargement of the liver. The eggs may form embolisms in various organs, most frequently in the liver, in which they cause inflammation and increase in the connective tissues, producing a type of cirrhosis in which the surface of the organ is coarsely and irregularly granulated. These changes assist in bringing about more or less prominent portal stasis. The eggs in the mucosa and submucosa of the intestine, especially of the colon, cause more or less severe inflammation; resulting in part in the destruction, in part in the formation, of tissue, changes which are sometimes followed by the tumor-like growths described by Kanamori, and sometimes by ulcers.

Katsurada believes that the disease originates from stagnant water. He says that in summer the water standing in the rice fields becomes covered with bubbles which break when in contact with the skin, with resulting itching and eruptions. Infection, then, he thinks takes place through the abraded skin. In places where artesian-well water is used and where the people do not wade in the bubble-covered water the disease is becoming less frequent.

Since visiting the farming districts of Japan I have little doubt but that the disease is a water-borne one and that it originates in the rice fields or irrigated gardens. The same is true of China. In both these countries the fields are fertilized by human excreta to such an extent that in many places traveling is most unpleasant because of the odor. Under such circumstances the opportunities are excellent for the transmission of a disease which is caused by a parasite the ova of which are

passed in the stools. Whether infection occurs through the skin or not is still a question, though from the distribution of the eggs in the body we would suppose that it occurred by the gastro-intestinal route. However, the same is true of uncinariasis, and still there appears to be considerable evidence of the occurrence of the latter infection through the skin.

The significance of this new case is evident. It means that not only in China and Japan but also in the Philippines there is a disease caused by a blood parasite which may of itself, or by its eggs, and perhaps also by a toxin, produce a serious condition resulting in cirrhosis of the liver, splenomegaly, ascites, dysentery, progressive anæmia, and also, possibly, epilepsy of the Jacksonian type. In certain stages of the infection the condition may be confused with tropical splenomegaly, of which it possibly is one of the much-sought-for causes; or with amœbic dysentery or uncinariasis, with either or both of which it may be combined, or with epilepsy. It is very probable, now that a case has been encountered, that further ones will be discovered, and perhaps it will be found to be nearly as common, both in China and the Philippines, as it is in Japan.

The following method of staining the ova in the tissue was devised by Mr. Willyoung, of the Biological Laboratory:

Celloidin sections were immersed in water and then stained in a solution containing 1 per cent acid fuchsin and 2 per cent oxalic acid. They were then washed in water and stained in an aqueous solution containing 0.12 per cent of aniline blue and 1.2 per cent oxalic acid. Differentiation was accomplished by using acid alcohol and 80 per cent alcohol. By this means the ova were stained a brilliant red and the tissue a clear blue.

SUMMARY.

In lesions in the lungs, liver, and the bowel of a Filipino, ova have been found which agree in shape, size, and color with those of *Schistosoma japonicum vel cattoi.*

The lesions in the bowel were ulcerations closely resembling those seen in some forms of amœbiasis; those in the liver were characterized by fibrosis.

The symptoms were not definite, because of the mixed infection with other intestinal parasites.

From these observations it follows that in China, Japan, and in the Philippine Islands there is a trematode worm differing characteristically in its morphology from the allied African species, which produces lesions, especially in the large intestine and liver, and which has been described as *Schistosoma japonicum vel cattoi.* The case under observation is, to the best of my knowledge, the first schistosoma infection encountered in the Philippine Islands, and, therefore, now that it has been called to the attention of investigators, it seems not unlikely that other cases will be discovered.

Since the above was written, a second Chinese case of schistosomiasis has been recorded by Bayer (*Amer. Med.* (1905), X, 578). This case was first observed by O. T. Logan, of Changteh, Hunan, China, who made clinical notes upon the case and who later sent these and specimens of the faeces to the Naval Medical School. The patient, a boy of 18 years, for six years had bloody stools. At 15 years of age he had been incapacitated for hard work. Logan found the liver and spleen enlarged, the latter but slightly. The stools, which continued to show blood, averaged about four in twenty-four hours and were preceded by abdominal pain. The ova of the parasite were found in the feces, and each ovum contained a ciliated embryo. Logan thought the ova were those of *S. japonicum*, and in this view Stiles, Lovering, and Beyer coincide.

From the following articles I have drawn very generously, and to Dr. Stiles and Dr. Shiga I wish to express my gratitude:

CATTO: Schistosoma cattoi: A New Blood Fluke of Man. *Brit. Med. Jour.* (1905), I, 11; *Journ. Trop. Med.* (1905), VII, 70.

SCHEUBE: Ein Neues Schistosomum beim Menschen. *Arch. f. Schiffs- und Tropen-Hygiene* (1905), IX, 150.

STILES: The New Asiatic Blood Fluke (*S. japonicum*, 1904; *S. cattoi*, 1905) of Man and Cats. *Amer. Med.* (1905), IX, 821.

KATSURADA: An Endemic Disease Caused by a Special Parasite Previously Unknown in Japan. *Sci. I. Kwai.*, XXIII and XXIV. (Review in *J. A. M. A.* (1905), XLV, 80.)

LOOSS: Schistosomum japonicum Katsurada, Eine Neue Asiatische Bilharzia des Menschen. *Centr. f. Bakt.*, Orig. (1905), XXXIX, 280.

ILLUSTRATIONS.

FIG. 1. Ova in the periportal connective tissue of the liver. Hematoxylin. (Photomicrograph.)

2. Ova in the interlobular perivascular connective tissue of liver. Hematoxylin. (Photomicrograph.)

3. Ova in the parenchyma of the liver lobule. Shows small-celled infiltration and commencing fibrosis. Hematoxylin. (Photomicrograph.)

4. Ova in lung. Hematoxylin. (Photomicrograph.)

5. Ova in mucosa and submucosa of large intestine. Shows atrophic and infiltrated condition of mucosa. Hematoxylin. (Photomicrograph.)

In all instances the photographs were made with the Zeiss photomicrographic apparatus, compensation ocular No. 6, objective AA; bellows at 45 centimeters.

FIG. 1.

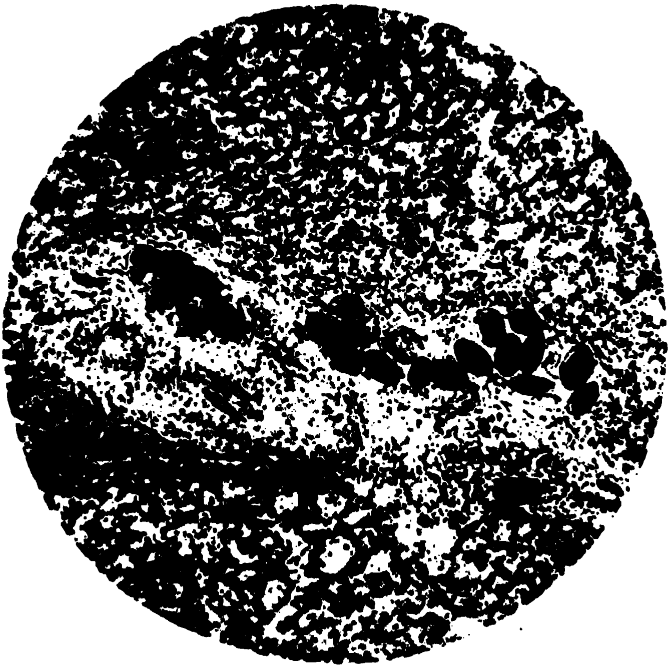

FIG. 2.

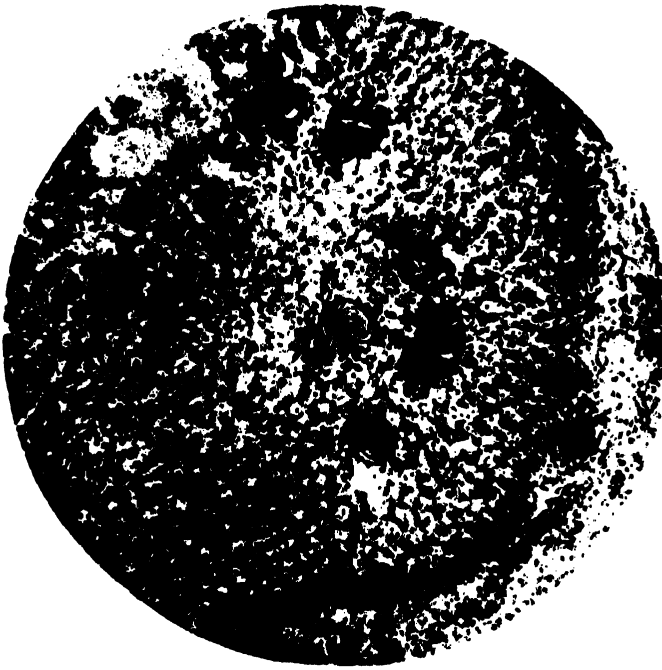

FIG. 3.

FIG. 4.

FIG. 5.

A STUDY OF SOME TROPICAL ULCERATIONS OF THE SKIN WITH PARTICULAR REFERENCE TO THEIR ETIOLOGY.

By Richard P. Strong.
(*From the Biological Laboratory, Bureau of Science.*)

In Manila subacute and chronic ulcerations of the skin, of obscure origin, are not infrequently encountered. During the past two years I have examined, particularly from an etiological standpoint, all forms of ulceration of this nature which have come to my notice. So far, twenty-four instances have been studied. Many of the lesions in these cases differed widely in their clinical manifestations, and I was able to demonstrate conclusively that at least several of them varied in their etiology.

It was originally my intention to review in detail the examination of each case; but on going over my notes I found so little which was characteristic or of interest in many of them that I have considered it more advisable here to discuss merely those instances which either proved etiologically to be or seemed clinically to represent distinct and specific infections. Thus, in a number of cases, a history of various primary injuries of the skin was obtained; and although at first it was intended not to include in this study ulcerations occurring in the course of certain chronic diseases, such as leprosy, syphilis, or yaws, nevertheless, of the lesions of seven of the cases investigated, five later proved really to be those of yaws and two of syphilis. Only staphylocci, streptocci, or saprophytic bacilli were isolated in the larger number of the lesions studied. However, in addition to those instances in which, both clinically and etiologically, nothing definite could be discovered, and to the ones which represented lesions in the course of those chronic affections I have mentioned, three distinct clinical types of disease were encountered, and these will now be considered.

ULCERATION OF THE FIRST TYPE.

In the first instance, the lesion to be discussed answers very well, both in its clinical appearance and in its history, to the usual description of *Delhi* or *Oriental boil,* as found in the several text-books on tropical diseases. The history of this case may briefly be recorded as follows: The patient was a native woman about 35 years of age. Approximately six weeks before consulting me she stated that she had noticed a small red

spot on the right side of the chest just below the clavicle and above and to the right of the breast. The lump gradually enlarged, but occasioned little pain. When I first saw the boil it was about the size of a half dollar and had not opened. The skin over the center was scaly, indurated, and reddened. Fluctuation could be obtained. On incising the abscess in the center, a scanty, rather thick, purulent material was found and a small ulcerating cavity, containing soft granulations, was exposed. On the following day, under cocaine anæsthesia, the whole cavity was carefully curetted. The operation was thoroughly performed, because the patient insisted on leaving on the next day for the provinces to be gone for several months. Directions were given for the daily antiseptic dressing of the wound. I was unable to see her again until four months later, when, after repeated requests, she finally returned to the city and exhibited to me a contracted scar the size of a dollar, situated over the original site of the lesion. She reported that the wound had gradually healed, about two months after leaving the city. Portions of the granulations which were removed with the curette were hardened in Zenker's fluid, embedded, sectioned, and stained in hematoxylin-eosin, hematoxylin-picrofuchsin, methylene-blue-eosin, fuchsin, Borrell's stain, and Wright's modification of Romanowsky's method. Figs. 1 to 10, inclusive, are photomicrographs made from these sections.[1]

The histological changes in the portions of the granulation tissue which were removed consist of a chronic inflammatory process in the subcutaneous tissue, with areas of acute inflammation, showing cellular infiltration and in places necrosis, together with considerable fibrin formation. In addition, the infiltration consists of numbers of cells whose protoplasm stains poorly, of fragmented nuclei, polymorphonuclear leucocytes, and small round cells with deeply staining round nuclei. In places the lymph spaces are widened, and there is an extensive proliferation of the endothelial cells of the lymphatic vessels, and in some areas these occur in rows, thus suggesting their origin from their arrangement. There is also considerable proliferation of the fixed connective-tissue cells. Multinuclear giant cells are occasionally observed and plasma cells are fairly numerous. In certain of the inflammatory areas eosinophiles are greatly increased in number and not infrequently many free eosinophilic granules may be seen, but only occasionally a mast cell is visible here. In the sections a striking feature is the presence of numerous large endothelioid phagocytic cells, with a relatively large amount of protoplasm and with a large round or oval nucleus, which may contain a nucleolus. Sometimes the margins of these cells are indistinct. No bacteria are evident in the sections. The presence of parasites, which are scattered throughout the tissue, is of chief interest. These may be described as oval

[1] I wish to express my thanks to Mr. Willyoung, of the Biological Laboratory, for his success and interest in the staining of the parasites in the tissues of this case.

bodies, which resemble cockleshells, with a sharp outline, measuring about 3 to 4 μ in their greatest diameter. In sections stained with hematoxylin and eosin they usually remain unstained; in those treated with picro-fuchsin, Borrell's blue, or Wright's method, they are still to a large extent uncolored, but many contain particles of chromatin, which stain and which consist, first, of a rounded mass, which sometimes has the form of a ring, and secondly, of a small dot or rod. These bodies are found in large numbers, both free and inclosed in endothelioid phagocytic cells, or lying in a sort of matrix composed probably of degenerating tissue. As many as ten or twelve may be seen in a single cell. They are very definite organisms and there is no doubt that they are parasites. In not all of the cockleshells are the chromatin masses present and many of them contain either the ring body or the pigment dot or rod alone (figs. 5 and 9). In fact, it is somewhat exceptional to see both chromatin particles in the same parasite; or, at least, either the one or the other body alone is in focus at one time. The shape of the chromatin masses also sometimes varies. Frequently, seal-ring-like forms are encountered (see figs. 7 and 10), or again at times a crescentic mass of pigment is seen situated alone at the edge of the ring. Such examples may be observed in figs. 3 and 4. With Zeiss objective DD and ocular 3 the organisms appear as small dots and oval bodies, often lying free, but generally inclosed in phagocytic cells. Their appearance under this magnification is illustrated in fig. 2. The further discussion of these organisms will be taken up below. Agar cultures which were made at the time of the curetting of the lesion remained sterile.

I do not wish to be misunderstood as insisting that the lesion in this case should be regarded as identical with Delhi boil, but merely to call attention to the fact that both in its clinical and in its histological appearance it has many points of resemblance to the latter affection. Unfortunately I did not obtain any skin immediately over the surface of the boil from this patient. A small portion which was secured from the edge of the lesion does not show any destruction of the papillary layer, though this shows cellular infiltration near the edge of the section. However, this process is more marked in the reticular and subcutaneous strata. As will be referred to presently, the diagnosis of Delhi boil is frequently extremely difficult since the affection presents so little that is distinctive and characteristic.

ULCERATION OF THE SECOND TYPE.

The second type of ulcer, when first seen, clinically differed very much from the one just described. The patient was a native man 25 years of age. The ulcer occurred in the region of the right shoulder, as may be seen from fig. 14. No history of injury to the skin or of trauma was obtained. The patient stated that a little over two months before I saw him, a small, red "spot" appeared over the right shoulder. This gradually enlarged, became hard to the touch, slightly painful, and finally a little fluid began to escape from the surface of the sore. Later the lesion became covered with a black scab. After this condition had

lasted for about six or seven weeks, during which time the sore slowly increased in size, he consulted a native physician, who first poulticed the area and then treated it with antiseptic dressings. About two weeks later he came to Manila for treatment, a diagnosis of tropical ulcer having been made, and he was referred by the Civil Hospital to the laboratory for examination. The lesion revealed the condition shown by fig. 14. There was no longer any scab covering the entire area, though here and there, over the surface of the lesion, there were a few hardened crusts, and in other places small patches of a pseudomembrane of a grayish color. These patches may be distinguished in the figure. In general, the surface of the ulcer was moist and covered with a yellowish-gray, purulent exudate. The base was very uneven and was covered with areas of necrotic tissue or with fresh granulations. The edges were undermined. In depth, the lesion extended through the entire skin and into the subcutaneous tissue and in places the muscle was just exposed. Dr. Cook, of the Civil Hospital, has kindly informed me that after curetting and antiseptic treatment, this ulcer, finally, after three months, healed and the patient was discharged, cured.

The lesion, in this case, when first observed by me, seemed to correspond very well with the description of "tropical sloughing phagedæna," as it is described by Manson, although the physical condition of the patient was otherwise good and the development of the ulcer did not occur exactly as Manson describes it. It is perhaps unnecessary to add that the lesion in this case was not syphilitic.

Film preparations from various portions of the ulcer were made on cover glasses, and these were examined both in a fresh condition and after drying and staining. While numerous, short, thick bacilli and a few cocci were found to be present in these films, nothing which suggested protoza or other parasites was observed. No tubercle bacilli were present. Portions of the tissues were excised for histological study, and agar plate cultures and bouillon tubes were prepared from the lesion, the oese being pushed through the soft granulations and also beneath the overhanging margins of the skin. After twenty-four hours the plate cultures developed numerous colonies, the great majority of which closely resembled one another. In fact, the cultures were almost pure. Colonies occurred on the surface and in the depth of the media. Usually most of those on the surface were round, whitish-gray, and moist. Under a low magnification they were often nucleated and their margins were frequently uneven. There was little which was characteristic about them. Microscopical preparations, which were made from a number of colonies, showed the organism to be a short bacillus, measuring about 1.5 μ in length and about 0.6 μ in breadth. Its motility was only moderate, although later, numerous flagella were demonstrated. When inoculated in gelatin, rapid liquefaction of the medium occurred, with hair-like projections in the line of the stab. Litmus milk was very slowly coagulated.

The reaction was but little changed. Glucose and saccharose were fermented but not lactose. Indol was rapidly produced. The growth on potato was abundant, grayish, and moist. The other colonies, which had a different appearance and which developed on the plate cultures, proved to be those of *Staphylococcus aureus;* they were not by any means so numerous as those of the bacillus just described. Two guinea pigs were inoculated intraperitoneally and one rabbit intravenously, each with one-third of a twenty-four agar slant culture of the bacillus, suspended in saline solution. The animals remained alive and no pathological effects were noted. A small area of the skin over the abdomen of a monkey was shaved and the animal inoculated subcutaneously with 1 cubic centimeter of the bouillon culture from the human lesion. This culture was known not to be pure; it contained, besides large numbers of bacilli, a few cocci. The animal died from asthenia three weeks after the inoculation. It had been long in captivity and was somewhat emaciated at the time of its death. At autopsy, cultures taken from the heart's blood, liver, and spleen all remained sterile. On the abdomen, near the point of inoculation, was a small nodule, measuring about 1 centimeter in diameter, over which the skin was reddened and almost perforated. On incising this area an ulcer, the edges of which were ragged, infiltrated, and undermined, was found in the subcutaneous tissue and corium. A small amount of pus was present. Many cultures from this lesion developed a large number of colonies of *Staphylococcus aureus*, but only a few of the bacillus above described. This latter organism was regarded as a variety of the *Proteus* group. On account of its apparent nonpathogenicity for animals, no further attempt was made to identify it more closely.

The tissues from the human lesion were hardened in Zenker's solution and stained in hematoxylin-eosin, methylene-blue-eosin, carbol fuchsin-methylene-blue, Weigert's stain, and Wright's modification of Romanowsky's method. A histological examination of the sections reveals, usually upon the surface of the ulcer, a dense homogeneous layer, in which the structure of the tissue can no longer be recognized and which stains diffusely red with eosin. Scattered here and there through this mass may be seen numbers of polymorphonuclear leucocytes and in places a large amount of fibrin can be detected. In the upper portion of this necrotic tissue the bodies of the cells do not stain at all, the polymorphonuclei in many instances appearing as if they were lying in clear vacuoles; in other cases the cell protoplasm is stained partly or wholly light pink. A little deeper in the tissue the number of polymorphonuclear leucocytes is greatly increased and the protoplasm of these cells stains well with eosin. Many red-blood corpuscles as well as many fine threads and coarser fibrils, lying between the cells, can be distinguished. These fibers, in specimens stained by the Weigert method, are seen to be fibrin. In other places, in this portion of the tissue, a dense coagulation of the liquid exudate (evidently first secreted) has also taken place, in which

the fibrin masses and cells may be distinguished. The process near the surface may therefore be said to represent an extensive coagulation necrosis. When the lesion is examined with the naked eye, these homogeneous necrotic masses of tissue, in which much fibrin is deposited, give rise over portions of the surface of the ulcer to the very striking pseudomembranous appearance, already referred to. Deeper still in the section the connective-tissue cells are seen to be very greatly proliferated and in places plasma cells are plentiful in number. In other portions of the tissue, the connective-tissue fibers are pushed apart, and there are other evidences of inflammatory œdema. Between these fibers may be seen a few small round cells, red-blood corpuscles, and polymorphonuclear leucocytes. The condition of the blood vessels in which there is a considerable proliferation of the perithelial cells, is quite striking in these areas of inflammatory œdema. The process is particularly well marked about the veins. In many of the vessels the polymorphonuclear leucocytes within their lumina are increased in number, and sometimes these may be seen lying between the several layers of the meso and perithelial cells, which partly form the wall of the vessel. Near these areas large multinuclear giant cells are occasionally observed. Very few eosinophiles are present, and only occasionally a plasma cell is here seen. Scattered through the section are numerous other areas of necrosis, in which, generally, considerable fibrin is present and in which there are also numerous red-blood cells, small round cells, polymorphonuclear leucocytes, fragmented nuclei, much granular material, and many bacteria. Eosinophiles are not seen in these situations; but bacilli, which only partly decolorize by Gram's stain, and cocci, which retain the latter, are encountered usually lying between the cells. No bodies resembling protozoa and no tubercle bacilli appear to be present. In a section at the edge of the ulcer, which includes some of the epidermis, the cells of the Malpighian layer are proliferated. In places, this layer is extensively infiltrated and sometimes apparently destroyed, the area of infiltration and necrosis reaching from below upward to the corneal layer, which it touches. Evidently in this way new foci of ulceration are formed. In certain areas the corneal layer has entirely disappeared. In these places of necrosis an appearance similar to that which has been described in regard to the subcutaneous tissue is seen—coagulation necrosis, deposits of fibrin, fragmented polymorphonuclear leucocytes, etc. The papillary and reticular layers are also extensively infiltrated with round cells and polymorphonuclear leucocytes; in addition both of these strata contain areas of necrosis similar to those already described. The histological appearances are pictured in figs. 15 to 18.

The lesion in the monkey histologically consists of an abscess cavity in the corium and subcutaneous tissue, about which there is little which is characteristic. The pathological changes encountered appear in many ways similar to those seen in the human lesion; but the process is less chronic and there is no proliferation

of the cells about the blood vessels. Cocci and a few bacilli, together with much granular material and many degenerating cells, are distinguishable in the abscess cavity.

As a result of this study the etiology of the human lesion in this case must remain obscure, at least, so far as the discovery of any single specific organism is concerned. Attention may again be called to the fact that no protozoa were encountered in the sections. That the *Proteus bacillus* and the *Staphylococcus aureus*, occurring in symbiosis, alone were responsible for the original lesion seems unlikely. However, there can be little doubt that these bacteria modified and caused an extension of the disease process. It is possible, even if not probable, that the organism responsible for the initial lesion in this case had already disappeared from the ulcer when I had the opportunity of examining it.

ULCERATION OF THE THIRD TYPE.

The third type of ulceration was observed in three cases—the first in a teamster, the second in a mechanic employed in an iron foundry, and the third in a male nurse. All of these were white men.

Although I have been unable to discover the specific organism for this affection, it evidently varies both in its origin and nature from the two forms of ulceration already described. For this reason, as well as on account of the peculiar type of the lesions, it has been thought of importance to call attention to them and to describe the cases somewhat in detail. In all three the ulcerations were multiple and were situated on the hands and forearms. In only one of the cases did the lesions occur elsewhere; in this instance they were situated on the feet and ankles; although they were not present here at the time I was able to study the case. Figs. 19 and 20 very well represent the distribution of the lesions in two of the cases. There were no general disturbances and there was very little or no itching. The lymphatic glands in the region of the elbow were the only ones which were swollen, and these were very slightly enlarged. One of the most striking features of the affection is its extreme chronicity. The disease usually commences by the formation of several small vesicles, which sometimes break and later form superficial ulcerations. In other cases, the vesicles become pustular before opening through the surface of the skin. Areas of fresh infection seem to occur from the older lesions, though I have not been able to entirely verify this fact from a microscopical study of sections. The ulcerations, as a rule, are shallow and but slightly painful; their margins are smooth; they do not have a punched-out appearance and they are not undermined. Their edges are reddened but not indurated. The skin for about 1 or 2 centimeters surrounding the ulcer is also erythematous. There seems to be little tendency for thick crusts to form, but small, soft, yellow scabs are frequently seen scattered over the surface. There is but little discharge; when it occurs it is usually of a serous nature. After several

weeks or months the ulcerations gradually heal; but others quickly form in the adjacent areas of the skin. In one of the cases the lesion persisted for a year and a half, in another for nine months, and in the third (see fig. 20) for nearly three years. Nodules which have not yet broken down, shallow ulcerations, and scars of old lesions may be distinguished in the photograph. In addition to the ulcerations, there are usually to be seen nodular thickenings of the skin and subcutaneous tissue, which have not yet broken down. The skin over the unbroken nodules is reddened. Occasionally these nodules become covered with scales for some time before opening; when they ulcerate, there is only a very small amount of pus present. Potassium iodide and mercury seem to exert no effect on the course of the disease.

Cultures from the ulcerations of two of the cases developed pure growths of *Staphylococcus aureus*, while those from the third showed colonies both of this organism and of those of *Staphylococcus albus*. In one of the cases cultures were taken from an unbroken nodule and these remained sterile. Therefore, the infection does not appear to be of bacterial origin; though it must be emphasized that I was not able to inoculate any cultures in the vesicular stage. The nature of the lesions suggests in some respects that they are blastomycetic in their causation. However, none of these organisms could be discovered in sections.

In one of the cases the histological examination of a section from one of the nodules, which had not perforated the skin, shows in the subcutaneous tissue a cellular infiltration about a vein. The process consists of a true endo-, meso-, and peri- phlebitis. The proliferation may be seen beneath the endothelial layer of cells lining the vessel and extending outward into the surrounding subcutaneous tissue; it consists chiefly of endothelial cells, small round cells poor in protoplasm, and a very few plasma cells. Practically no polymorphonuclear leucocytes are visible and eosinophiles also are not observed outside the vessel; there is in addition early but extensive proliferation of the fixed connective-tissue cells.

As may be seen from fig. 21, the infiltration is eccentric and does not include the whole circumference of the vessel wall. A short distance from the vein the tissue appears normal. Several other foci of infiltration of a similar character are found in the subcutaneous tissue and one in the papillary layer, but none of these are about blood vessels. In sections stained by Weigert's method, no fibrin is demonstrable in these areas. There is no infiltration around the hair follicles or sweat glands and no marked œdema of the tissue. The papillary layer is in general unaffected. Indeed, the section, apart from the areas of infiltration already mentioned, appears normal.

In a section of tissue taken from near the edge of one of the ulcerations a small area of degeneration chiefly composed of degenerating cells of the mucus layer may be seen in the Malpighian layer. In many cases

only the distorted and deeply staining nuclei of these cells can be seen, the protoplasmic portions having disappeared. In addition, small round cells and a few leucocytes are present. No fibrin and no bacteria or other parasites can be detected. Beneath this area in the corium there is infiltration with small round cells. The microscopical picture is somewhat similar to that which Gilchrist has described in the papillary variety of *erythema multiformæ*. In other portions of the tissue in the region of the ulcer similar areas of degeneration may be found in the papillary layer of the corium. In a section of one of the ulcerations near its edge there may be seen on the surface a superficial layer of coagulation necrosis, in which fragmented nuclei and polymorphonuclear leucocytes are present. A few cocci may be distinguished in these areas. A small amount of fibrin can also be demonstrated. In places the necrosis does not extend below the corneous or the mucus layer, being entirely confined to the epidermis. In other portions of the section the papillary stratum is exposed, which then also shows inflammatory infiltration.

The lesions of this affection bear some resemblance to those described by F. Plehn[3] in his mild cases of "ulcerative dermatitis," but the distribution of the ulcerations is different. The disease seems to bear no resemblance whatever to the affection known as *chappa*, as described by Read, or to that of *Pian bois*, by Darier and Christmas.[4] Perhaps in the ulcerative stage it might be considered as one of *veld sore*,[5] in which secondary infection has occurred, but until its etiology is discovered, it is difficult to classify such an affection. By some observers it might perhaps even be considered as belonging to the type known as *Oriental sore*.

CONSIDERATION OF THE ETIOLOGY OF DELHI SORE OR BOIL.

During the past few years the study of one form of tropical ulcer has assumed renewed interest, chiefly, perhaps, owing to the discovery by J. Homer Wright, (1) in 1903, in a case of *Delhi boil*, of certain bodies which have considerable resemblance to the organisms already described by Leishman and Donovan in cases of tropical splenomegaly. While, in general, the clinical descriptions of this type of tropical ulcer (*Delhi boil* or *Oriental sore*), as found in several of the text-books on tropical diseases agree quite closely, when one examines into the clinical features, as set forth in the individual papers of those who have made special studies with reference to the etiology of this form of ulcer, considerable differences in the descriptions are found. Indeed, in many of these articles the reports of the macroscopical appearances of the lesions vary so widely that one is almost led to conclude that more than one type of the disease has been described under the name of *Delhi boil*. Such an opinion is not

[3] *Die Kamerun Küste*, Berlin (1898).
[4] *Ann. derm. et d. syph.* (1901).
[5] Harman, *Journal Pathology and Bacteriology* (1903).

entirely new. Geber, (2) as early as 1874, doubted that such a disease *sui generis* existed, and he further maintained that there was much abuse of the term "*Aleppo boil*," because lupus, scrofulous, and syphilitic lesions were frequently described under this name. The statement of James, (3) one of the most recent contributors on this subject, is also suggestive of the idea above given. He expresses the opinion that the appearance of some true *Oriental* or *Delhi "sores"* is by no means as characteristic as one would expect it to be from the descriptions given in books. Indeed, James found that several surgeons whose experience with the disease was extensive, were unwilling to express a definite opinion as to whether a given sore was really *Oriental sore* or whether it was an example of the ordinary chronic ulcer so common among the natives of India. He emphasizes the fact that *Oriental sore* does not always present very definite characteristics and states that in two of his cases the diagnosis was at first mistaken, the sore in one instance being considered primarily as a form of ringworm and in the other as an ordinary "*shoebite*." The gross appearance of the lesions in a number of the cases which James studied etiologically also varied widely.

Plehn, (4) in his very recent article on this subject, calls attention to the fact that it has not been thoroughly established that the symptom-complex described by the various authors and observers in different regions under the name of "*Beulenkrankheit*" represents a single distinct affection. According to him, some of the descriptions of the lesions might apply to those of furunculosis or of tertiary syphilis or of lupus, and he feels convinced that such errors in diagnosis in the ulcerative stage of the lesion, have certainly occurred frequently. Plehn further comments upon the fact that only in Jeanselme's recent article is framboesia considered in the discussion of the differential diagnosis, and although he emphasizes the fact that he does not consider the two affections identical, nevertheless, he believes that in their external appearances as well as in their histological structure they in some respects show so great a similarity that any one who is familiar with only one of the affections could occasionally mistake it for the other.

Jeanselme (5) in his article states that, while the *bouton d'Orient* may have a typical aspect and evolution, numerous clinical varieties may also exist. These he describes, and in the discussion of the differential diagnosis of the affection, the lesions of syphilis, of lupus, of leprosy, and of yaws are considered. He also refers to the presence of a lymphangitis in association with the lesion which extends from the region of the ulcers and causes a swelling of the adjacent lymphatic glands. The normal condition of the glands in *Oriental sore* has usually been emphasized by other observers as an important symptom in the differentiation of this affection from "yaws." Kaposi's (6) and Duhring's (7) clinical descriptions of the malady vary so widely that some investigators have doubted whether these authors observed the same affection.

However, not only from a clinical standpoint do the descriptions in the literature differ, but also from a pathological-anatomical one, the reports of the histological appearances either showing considerable variation in the lesions, or, at least, nothing sufficiently characteristic to make an accurate diagnosis of the condition possible. Obviously, these descriptions might be expected to vary greatly in the different stages of the affection, since after the lesions have progressed from the "boil" stage to the ulcerative one and secondary infections have occurred and cicatrization has resulted, the appearances must differ considerably from those seen in their incipiency, and hence the diagnosis of the affection from a pathological standpoint is attended with additional difficulties.

Cunningham, (8) who in 1885 described peculiar parasitic organisms in the tissues of specimens taken from this disease, states that, apart from the presence of these organisms, the lesion presented no specific characters, but was essentially a simple granuloma, such as might arise in connection with the presence of persistent irritation dependent upon various causes.

Babes, (9) in his consideration of the pathology of *Oriental* boil, remarks that one is unable to distinguish the condition from an etiological standpoint, because the pathological changes are not sufficiently different from those frequently encountered in various other boils or furuncles.

Allusion has already been made to the similarity in the pathological conditions which Plehn refers to in yaws and *Delhi boil*, while in the articles of Riehl, (10) Leloir, (11) Unna, (12) Kuhn, (13) and Jeanselme differences in the histological changes are also mentioned; some of which, it is true (but probably not all), depend upon the stage in which the lesion was examined. Kuhn, in his article on the histology of "endemische Beulen," emphasizes the fact that but few characteristic changes were found, though it must be mentioned that from a study of the literature and photographs he concluded that endemic boil exists as a disease *sui generis*.

It is not my purpose here to enter into any further discussion of the literature in regard to this question, since the references given above will, I believe, convince the reader that confusion has occurred in the diagnosis of this disease, and it is more than likely that several different affections have been described under the terms *Delhi, Aleppo, Biskra, Gafsa boil, oriental bouton* or *sore, tropical ulcer, Pendjeh, Yemen, Sarten ulcer*, etc.

One might then be prepared to expect that many specific organisms should have been reported for this type of ulcer, and indeed such is the case. However, many of these observations have to-day only a historical interest or value.

In 1868 Smith (14) believed that he had succeeded in finding, in the sections of a tropical ulcer, ova of a species of distoma. Fleming, (15) in 1873, also thought that he had encountered the eggs of some parasite in the tissues from a case of *Delhi boil*. However, later he attributed another significance to the

bodies which he had mistaken for ova. Carter (16) in 1875, found in an ulcer of the lymphatic spaces of the corium, the mycelium of a fungus which contained many spores and orange-colored granules. However, as other observers have remarked, Carter's specimen had been kept for a long time in a weak preserving fluid which contained no alcohol and in which it probably became contaminated with the mold in question. In 1884, Deperet and Boinet, (17) in the study of an epidemic of *Oriental boil* among soldiers who returned from Tunis, cultivated a coccus from the lesions. This organism proved to be pathogenic for animals and upon injection produced nodules and ulcerations and sometimes a general infection. In the same year Duclaux and Heydenreich (18) also cultivated a micrococcus which they believed to be specific, from cases of *Biskra bouton*. This organism, when injected into animals in small amounts, sometimes produced a chronic lesion of the skin, which was said to bear some resemblance to that of *Biskra bouton*. In other cases the injection of this coccus caused the death of the animal within sixteen hours. Sufficiently accurate details for the identification of the organism are not given.

In 1885 Cunningham (8) reported the discovery of peculiar parasitic organisms in a specimen of *Delhi boil*. The lesion, which was examined histologically, had been placed in alcohol immediately after its removal. The epidermis over the boil was still intact, there being no ulceration present. The organisms varied considerably in size and in form; in some cases they were circular, in others elliptical, and in others irregularly lobate. In the majority of the instances their contour was smooth, but in some it was of a more or less tuberculate character. In some specimens a very delicate cell wall was clearly visible; in others it was wholly unrecognizable, or only to be detected on careful and special scrutiny. Cunningham further states: "The distinctness with which they appear in sections treated with Gentian violet is due to the elective staining by the dye, of the nucleoid bodies which they contain. The number of such bodies present in different cells is extremely variable. The cytoplasm in the gentian-violet specimens remains almost uncolored; in those in which fuchsin has likewise been employed it frequently shows a more or less pronounced red hue. The tuberculate appearance presented by some of the cells is due to the numbers and size of the nucleoid bodies present in them, which in association form a mulberry-like mass pressing upon the cell wall and molding it to the inequalities of its surface. In certain cases appearances apparently corresponding with the occurrence of processes of cell division are present, the bodies of the cells being strongly constricted so as to form two lobes connected by a narrow neck, or two distinct cells occur which, from their relations to one another and the character of their opposed surfaces, seem to have just arisen and to be due to the completion of such a process. The individual cells in some cases are closely packed among the surrounding lymphoid elements; however, in a large number of instances, they appear to lie in a limited clear space. The number of cells visible in individual sections and in different parts of the same section varies considerably. Entire fields may in certain places fail to show any at all. This failure may in many instances be due to imperfect success in staining, but, allowing for this, there can be no doubt that the numbers present in different parts of the tumor vary greatly. It is only quite exceptionally that any are present in the epidermal stratum. The continuous stratum of granulation tissue beneath the papillary layer is the site in which they occur in greatest quantity, but specimens are also frequently present in considerable numbers within the papillary eminences. Their distribution is not limited to the epidermal and dermal strata, for on passing downward to the subcutaneous tissues scattered specimens may be found in the very deepest parts." Cunningham is inclined to regard these bodies as representing various

stages of the development of some simple parasite of mycetozoic nature and concludes that they probably belong to the group of *Monadinæ*. However, he expresses the opinion that it is impossible to come to a definite conclusion as to their nature or to the relation which they bore to the disease.

As Wright (1) has remarked: "From Cunningham's description of these bodies the morphological evidence adduced in favor of their parasitic nature is not sufficient to overcome the objection that they are elements of the tissue or degeneration products."

Nevertheless, while it is true that Cunningham's illustrations do not definitely show that the bodies in question are parasitic in nature, they are equally or even more convincing of the presence of parasitic bodies in *Oriental sore* than are a number of the drawings in some of the very recent articles on this subject.

Riehl, (10) in 1886, found in a single case of this disease a capsulated micrococcus which occurred particularly in the cytoplasm of large epithelioid cells. As many as twenty of these organisms were encountered in a single cell. Cultures from the lesion developed no growth. In the same year, Loustalot and Leloir (19) cultivated a micrococcus which they considered specific, but which Leloir later concluded to be only a variety of the common *Staphylococcus aureus*. Neuijmin (20) also in 1886 found in sections and nodes of 104 cases of *Pendjeh ulcer* a micrococcus which occurred singly, in pairs, or in short chains. No specific characteristics for the organisms were detailed.

Finkelstein, (21) in 1887, in three cases of *Pendjeh ulcer*, and Chantemesse, (22) (1887) in a case of *Nile ulcer*, also cultivated cocci which were believed to be similar to the organism described by Duclaux. In Chantemesse's case the boil had not perforated at the time of the examination. He inoculated a man by piercing the skin with a needle infected with the coccus obtained from the lesion in culture. After five days an abscess formed at the point of inoculation. Two days later this opened and a small, round, crater-like ulcer was exposed, which healed after treatment with antiseptics for some days. However, the organism cultivated from the lesion of Chantemesse's case showed but slight variations from some strains of *Staphylococcus aureus*.

Poncet, (23) also, in the same year, found in sections of a case of *bouton de Gafsa*, two species of bacteria, one a micrococcus and the other a long, thin bacillus. The coccus stained by Gram's method, but the bacillus became decolorized.

In 1888 Heydenreich (24) published his investigations upon a series of twenty-seven cases of *Pendjeh ulcer*, in which he concluded that the disease was caused by the *Micrococcus biskra*, which, together with Duclaux, he had already described in 1884. This organism was said to possess a capsule and to produce spores. Both the coccus isolated by Heydenreich and the other organisms isolated by the various observers prior to the time of his report are to-day believed to be merely species of *Staphylococcus aureus*.

In 1888 Raptschewsky (25) was unable to confirm the results of Heydenreich on the etiology of *Pendjeh ulcer* in cases which he studied and which came from the same region. Instead, he cultivated from the lesions a *Streptococcus*, and, sometimes in association with this, *Staphylococcus aureus*.

In 1894 Le Dantec and Auche (26) also found, in an ulcerated case of *bouton de Biskra*, a *Streptococcus* and *Staphylococcus albus*.

In 1897 Nicolle and Nourry-Bey (27) in nine cases of *Aleppo boil*, some of which had not perforated the skin, found in the blood and pus a streptococcus which they believed to be specific, particularly because of its reaction with Marmorek's serum.

Usually the organism exhibited but little virulence in animals, and the authors were unable to communicate the disease, even to monkeys. In three cases they also encountered staphylococci in association with this organism, and in one each a bacillus and a streptothrix, respectively.

In the same year, Brocq and Veillon (28) cultivated, from a case of *Aleppo boil*, a streptothrix, which, according to Legrain, was similar to the cladothrix of Madura foot. Inoculation into man was without result.

Djelaleddin-Moukhtar (29) also found a streptococcus in a case of *Aleppo boil* which he believed to be identical with the streptococcus of erysipelas.

Crendiropoulo (30) encountered in Camaran, in numerous cases of the Yemen ulcer,[a] a small bacillus together with different saprophytic organisms and pyogenic cocci. A detailed description of the organism is given in his article. It was pathogenic for rabbits and doves, in large amounts causing septicæmia and in small amounts local infection and ulcerations which contained but little pus. Babes concluded that this organism probably belongs to the *Proteus* group of bacteria. Such bacilli he frequently encountered in chronic ulcerations of the skin in connection with the pyogenic cocci.

In 1891 Firth (31) claimed that he had also found, in the lesions of *Delhi boil*, the bodies described by Cunningham. He proposed the name *Sporozoa furunculosa* for the parasite, although he did not give any more distinctive proof than Cunningham that the bodies which were encountered by him were really of a parasitic nature.

In 1898 Borowsky (32) in the study of twenty cases of *Sarten ulcer* constantly found in the secretions and in the ulcers themselves certain organisms which resembled protozoa. In the hanging drop, the parasites had an active motility and were spherical or spindle shaped. They measured from 0.5 to 3 μ in size. The cell body stained but faintly. The nucleus was placed eccentrically. In dried preparations the organisms were very numerous. Frequently they were encountered within the lymphoid cells and red corpuscles. In sections the parasites were so numerous that sometimes their boundaries could not be distinguished. Only the nucleus, which stained well with Loeffler's methylene-blue, could be differentiated. Accumulations of the parasites also occurred outside of the cells. They then appeared as a group of round bodies with faintly stained protoplasm and eccentrically placed nuclei. Borowsky was not successful in staining the chromatin bodies nor did he succeed in cultivating the parasite in artificial media.

Schulgin, (33) in 1902, examined fourteen cases of this disease and confirmed the conclusions of Borowsky. He believed that the parasites multiplied by division and that he could distinguish young forms of the organism in the tissues. He also suggests that the disease is conveyed by the bites of mosquitoes.

In 1903, as mentioned above, Wright, (1) in the study of a case of tropical ulcer which occurred in a child from Armenia, found certain bodies which bear a resemblance to the so-called Leishman-Donovan bodies. Wright carefully described these forms and proposed for them the name of *Helcosoma tropicum*. The organisms were generally round, sharply defined in outline, and from 2 to 4 μ in diameter. A large part of their peripheral portions was stained a pale robin's-egg blue, while their centers were unstained or white. A very prominent feature was the presence in each of the bodies of a larger and a smaller lilac-colored mass. The larger, about one-fourth or one-third the size of the body, was of variable shape but always formed a part of the rounded periphery; the smaller in some instances was round, in others rodshaped, and in the latter case was of a deeper

[a] By many observers the "*Yemen ulcer*" is regarded as identical with "Tropical sloughing phagedæna."

lilac color than the larger mass. It was usually situated near or at the blue-stained periphery of the body. The blue peripheral portions of the body were usually sharply defined from the central unstained part and sometimes showed small unstained areas. A few of the bodies were oval or elongate in form. This was thought to be due to distortion in making the preparation, because in thin sections of the tissue such forms were not apparent. In the thicker portions of the smears the central part of the bodies was stained blue as was also the periphery. These bodies were present in very large numbers in the smears, often occurring in aggregations, associated with a large nucleus, thus suggesting that they had been contained in a large cell whose outlines had disappeared in the process of fixing and staining.

Microscopical examination of paraffin sections of some of the material which had been fixed in Zenker's fluid, showed that the micro-organisms were generally closely packed together throughout the cytoplasm of large endothelial cells with single vesicular nuclei. These large cells were very numerous over extensive areas and constituted the principal part of the infiltration. The organisms occupied most of the available space between the nucleus and the cell membrane. Many of these cells contained 20 or more micro-organisms.

A portion of the lesion of the ulcer was used for the inoculation of a rabbit by subcutaneous injection and by scarification of the skin and cornea. No pathogenic effect was noted in the animal. An attempt to cultivate the organisms in freshly drawn human blood was unsuccessful.

About the same time that Wright reported the results of his study, Marzinowsky and Bogrow (34) (1904) described the occurrence of somewhat similar bodies in a case of *Pendjeh ulcer* from Persia. They believed these bodies to be protozoa. They were encountered in smears from the granulations at the base of the ulcers and were usually oval in form, more seldom round, measuring from 1 to 3 μ in size. They were frequently found in the protoplasm of epithelioid cells; less often they were seen free. They were not observed in red cells. In the secretions of the ulcer, or in the old or healing ulcers, the parasites were either very scanty or absent. In hanging-drop preparations the organisms within the cells were not motile. When lying free they exhibited a slight progressive motion. In staining with the usual aniline dyes the entire body became colored and the nucleus could not be differentiated. Sometimes the bodies lay singly or several were grouped together in the vacuoles of a cell. If the preparations were stained after Giemsa's method for chromatin (methylene-azure and eosin), the structure of the body was more clearly differentiated. The entire body was stained blue, showing two particles of chromatin (macro- and micronucleus). The first, a larger mass, was rounded and stained light blue; the second colored more deeply (red-lilac) and usually appeared in the form of a rod; it was rarely spherical in outline. This latter body, when rod shaped, lay either perpendicular or parallel to the more lightly stained chromatin mass. In some of the forms only this rod-like particle of chromatin was stained. Attempts to cultivate the organism in various culture media failed. Experiments in the inoculation of rabbits and guinea pigs were also unsuccessful.

Plehn, (4) during the present year, has described in detail the lesions from a case in which the sore was contracted in Mesopotamia or south Persia. The epidermis over the lesion was unchanged. Upon microscopical examination, everywhere in the neighborhood of the area in which cell infiltration occurred, but here only and with increasing density toward the surface, could be seen, with a moderate magnification, collections of rounded bodies, measuring from 1 to 1.5 μ in diameter and lying between the round cells. With a higher power (apochromatic one-twelfth, compensation ocular 8–12) it could be observed that

these bodies were partly inclosed in epithelioid cells, the nuclei of which sometimes appeared to be pushed to one side. In other places, where they apparently were not enveloped in cells, the bodies lay in groups in such a manner that one might believe that they were lying inclosed in the same envelope, but that optically the latter could not be distinguished. The bodies themselves existed, first, as a deeply stained round or more elongated chromatin granule; and secondly, one about double the size, seldom three times as large, and of a somewhat different appearance. This second body probably representated a protoplasmic form, which in the central portion was lightly stained and at the margins was more deeply colored, so that sometimes a ring form resulted, at the periphery of which the deeply stained round body was situated. In a favorable light not infrequently there could be seen a second, very small, deeply stained round or more elongated granule, which either was attached to the larger one or which also appeared in the periphery of the ring, opposite to the larger body. The entire form sometimes seemed to be inclosed in a round or oval halo, which was either stained or remained uncolored.

Plehn remarks that, as the description demonstrates, the similarity of these forms to Leishman's bodies is very great; at any rate, we have to do with the occurrence of protozoa in *Oriental boil*, and the peculiar nature of these organisms makes it probable that they are the specific cause of the disease.

James (3) (1905) has very recently examined 18 cases of "*Delhi sores*" and found, in all of these, peculiar bodies which he believes to be parasites. The bodies were found within large endothelioid cells and under a low power appeared as micrococci in the protoplasm of the cell. A large number were also scattered through the films or sections, which were not inclosed in cells. When examined under a high power, these bodies, which resembled micrococci, possessed a very definite appearance and structure. Most of them were then seen to be oval in shape, but slightly broader at one end than at the other. However, a good many were quite round and some were pointed at both ends. The bodies varied considerably in size, but the length of the majority was about one-half the diameter of a red corpuscle. Their circumferences were remarkably regular and distinct, as if they were provided with a definite capsule. The greater portion of their substance stained a light blue, but near the center there was a large unstained area, sometimes divided into two by a streak of blue-stained body substance. In the interior of each body two masses of chromatin were seen. One of these was large in size, more or less rounded in shape, and was usually situated near the center, but always touching one edge of the circumference. The second chromatin mass varied in shape from a dot to a comparatively long, thick rod. In the latter case it usually lay near the center and at right angles to the long axis of the parasite. It stained more deeply than the large chromatin mass. In some parasites James saw a third, rod-shaped mass of chromatin, usually situated near the more pointed end of the former and at right angles to the second. This was present in only a few of the bodies in each film.

On the other hand, several competent observers, who have carefully examined specimens supposedly of this disease, have encountered no specific parasites.

Thus Unna, (12) (1894) who made a very careful histological examination of a specimen of *Delhi boil* sent from Constantinople by During, was unable to find any bacteria or other parasitic organisms in his sections. In a section from Riehl's case he found micrococci in enormous numbers within the necrotic cell masses, but they were not intracellular as Riehl had described them to be, all of those which Unna could distinguish being intercellular. Leloir (11) after diligent search was

also unable to find any parasitic organisms in the tissues. In one section only he encountered a single diplococcus, lying free between the infiltrating cells. In 1897 Kuhn, (13) who examined a specimen of endemic boil, which also had not perforated the skin and over which both the stratum corneum and the stratum Malpighium continued uninterrupted, was unable to find any protozoon-like organisms, only large and small cocci, occurring either in chains or in clumps, and short, thick rods, being observed. Still more recently (1903) Babes, (9) in the examination of sections from a case of *Biskra boil,* was unable to discover the presence of any bacteria or protozoa. He concluded that the descriptions of the histological examinations of other observers show little which is characteristic, and that they even differ considerably from one another. Further, he believes that we must still consider the etiology of *Aleppo boil* as unknown, and that the lesions probably arise from infections resulting from insect bites or represent certain syphilitic nodules and ulcerations.

Jeanselme, (5) still more recently (1904), has made a careful study of a case of *Biskra boil* which the patient contracted in Algiers, and states that the fixation of the tissue in this case was perfect. Nevertheless, although a careful and detailed description of the histological appearances is given, no mention is made of the presence of any organism to which the origin of the boil might be ascribed.

Finally, Bently (41) who examined in Assam over sixty cases of sores and ulcers resembling *Delhi boil* never encountered any bodies which suggested protozoa.

It will be seen then from this review of the literature that, of the etiological factors which have been described for *Oriental boil* or *ulcer,* no single species of bacteria can be regarded as the sole specific cause of this disease. Doubtless, the pyogenic cocci or even varieties of the *Proteus* bacillus may have been responsible for the causation of many of the lesions, or at any rate, partly responsible for the pathological changes. Possibly, violent scratching of certain insect bites and secondary infections with such bacteria may have been the exciting agents of many of these ulcers. Finally, it is not clear that a number of the reported cases of *Delhi boil* do not really represent certain lesions of syphilis and yaws. However, on turning from this class of cases, we find that in a number of other instances organisms other than bacteria have been considered to constitute the origin of the disease. As already mentioned, Cunningham was the first to describe peculiar parasitic organisms, which he considered to be protozoa, in the lesions. Firth next reported the discovery of similar bodies and proposed the name of *Sporozoa furunculosa* for them. The encapsulated cocci of Riehl, it seems, should hardly be considered as related to the bodies described by these two observers, particularly if one recalls the examination and report which Unna has made of one of Riehl's specimens.

No further reference in the literature to the presence of protozoa in this disease is found until 1898, when Borowsky believed that he had encountered such organisms, Schulgin in 1902 confirming his results. Finally, Wright in 1903, Mazinowsky and Borgow in 1904, and James and Plehn during the present year, have all reported the occurrence in the lesions of bodies which they believe to be protozoa. The descriptions

bear a very striking resemblance to those which have been given of the Leishman-Donovan bodies. Regarding the researches of Cunningham and Firth, Wright has already remarked that we can not be sure that these observers were encountering parasites. In considering the more recent work, extending from the investigations of Borowsky to the present time, it is very difficult in some instances to determine the exact nature of the bodies encountered. While sometimes the detailed descriptions of the parasites are very definite, yet, when the drawings or photomicrographs accompanying the articles are examined, grave doubts enter as to the nature or the identity of the bodies in the particular instance consulted, with those forms described by the other observers. I believe that it will be impossible for us to elucidate this matter from a consultation of the literature only; and I therefore think it will be advantageous to have histological specimens from all of the reported lesions in which the protozoon-like bodies have been encountered examined by one thoroughly competent observer who is willing to undertake this work.

On account of the similarity of the organisms encountered in these cases of *Oriental sore* to the so-called Leishman-Donovan bodies, let us consider for a moment something of the nature of these latter forms.

THE LEISHMAN-DONOVAN BODY.

As is now well known, Leishman, (35) in May, 1903, "in making smear preparations from the spleen pulp of a case of so-called dum-dum fever, was struck by the curious appearance among the spleen cells and red corpuscles of enormous numbers of small round or oval bodies, two to three microns in diameter, which corresponded to nothing which he had previously met with or had seen figured or described. They stained faintly with methylene-blue and with hæmetein, showing with these stains a sharply contoured or oval shape, but no detailed structure; but on staining them by Romanowsky's method, they were found to possess a quantity of chromatin, of a very definite and regular shape, which clearly differentiated them from blood plates or possible nuclear detritus. This chromatin appeared in the form of a more or less definitely circular mass or ring, applied to which, although apparently not in direct connection with it, was a much smaller chromatin mass, usually in the form of a short rod, set perpendicularly or at a tangent to the circumference of the larger mass. The outline of the sphere or oval inclosing these masses of chromatin was only faintly visible by this method of staining. These little bodies were scattered freely among the cells, as a rule isolated one from the other, but here and there aggregated into clumps composed of 20 to 50 members."

Leishman was unable to say what these bodies were at the time, but later, when working with nagana, upon investigating the blood and internal organs of a white rat, dead of this disease, he found bodies practically identical in shape and staining reaction with those he had encountered in the spleen of his case of dum-dum fever. He concluded that these parasites were degenerated trypanosoma and that probably this particular case represented an infection with this organism.

Donovan (36) was the next one to observe these bodies, and later Ross (37) and Laveran (38) also reported in regard to the parasites. Ross inclined to the belief that they were *Sporozoa*, while Laveran, who found

them inside the red-blood cells, concluded that they belonged to the genus *Piroplasma.* Later, Marchand and Leidingham, (39) Manson and Low, (40) Bentley (41), and Christophers, (42) Castellani (43) and others, all contributed cases of dum-dum fever, kala-azar, or splenomegaly, infected with these parasites. Marchand and Leidingham inclined to the original idea of Leishman that they were of trypanosomal origin. Finally, Leonard Rogers (44) and Chatterjée (45) have stated that trypanosoma have developed in their cultures of the Leishman-Donovan bodies. These parasites have been found in the spleen, liver, bone-marrow, intestinal ulcers, lymph glands, and, according to Laveran and Donovan, within the red-blood cells.

As we have seen, similar, if not identical, forms have been encountered in a number of cases of *Oriental sore.* And in addition Donovan and Christophers have found these bodies in small and large ulcers of the skin in cases of tropical splenomegaly; though Christophers emphasizes the fact that he never detected these bodies where there was no general infection with the parasite.

After carefully perusing all the articles of the various observers on this subject, and particularly on comparing their different illustrations, the questions arise: Are the bodies described in all cases of tropical splenomegaly, *kala-azar*, and *Delhi sore* identical, and what is their origin and nature? Are they forms of trypanosomata; or are they piroplasmata or sporozoa, or some other form of parasitic life? These questions we are not at present in a position to answer. However, had good photomicrographs been prepared in all cases, as in Wright's report, the solution of some of these problems might have been made easier.

NATURE AND RELATIONSHIP OF THE BODIES ENCOUNTERED IN ORIENTAL SORE.

Wright, in his paper, makes no comment upon the question of the relation between his bodies and those described by Borowsky in 1898 and Schulgin in 1902. One can not be sure that they all were encountering the same forms. Leishman's paper appeared in 1903, after the publication of Borowsky's and Schulgin's articles, and doubtless after Wright's report had left his hands. Marzinowsky and Bogrow in their consideration of the subject, are not entirely convinced that Borowsky and Schulgin really encountered parasites in their cases. The latter authors described forms within the red-blood cells which Marzinowsky and Bogrow did not observe. However, they consider their organisms to be identical with those which Wright described, and they believe that, while the bodies they encountered were probably related to the trypanosomata, they showed noteworthy differences from them. From a comparison of the photomicrographs of the two articles, it is difficult for me to be sure that the bodies described by Wright and those by Marzinowsky and Bogrow are really

identical. Plehn considers that, if the parasites encountered in *Oriental sore* have any relation to trypanosoma, this has not yet been demonstrated.

Christophers, James, and also Rogers conclude that the organisms found in *Delhi sore* can not be distinguished by microscopical examination from those obtained from the spleen and other organs in certain cases of splenomegaly and *kala-azar*. However, James adds that since, on the other hand, the parasites met with in the Punjab are apparently capable of producing only the comparatively mild local disease known as *Delhi sore*, while those in Assam cause only the dangerous general affection known as *kala-azar*, many interesting questions are raised; as for example, whether the parasites of *Delhi sore* and of *kala-azar*, though obviously belonging to the same class, are of different species. He further believes that the evidence adduced points to the fact that, even when numerous parasites are present in *Delhi sore* on the skin for a long period, no general disease, such as *kala-azar*, results.

The parasites which I have encountered and described in the first case are clearly not identical with Wright bodies, as may be seen by comparing Wright's photomicrographs with those from my sections. As to their nonidentity with the bodies described by Marzinowsky and Bogrow, I can not be sure, as the photomicrographs of these authors do not very distinctly picture the forms they encountered. However, from their descriptions of these bodies I would suppose them to be different. On comparing my photomicrographs with the illustrations of James, differences are also seen to exist. However, Wright's bodies and those of James would hardly be considered identical, if judgment is to be made from the illustrations. The organisms encountered in my sections simulate some of the forms occasionally seen in Leishman's specimens, one of which is illustrated in fig. 11. However, when compared with the majority of his parasites (fig. 13), one sees very striking differences. The distinctions between Wright's and Leishman's bodies are also very evident; while in specimens of the forms, which Rogers has very kindly sent me, I could not certainly identify his organisms with those of Leishman. The bodies present in my sections simulate more closely some of those pictured by Marchand and Leidingham in their recent article; but it is doubtful whether they are identical with these. Plehn's article contains no illustrations.

Christophers noted that the bodies which he encountered had a very sharp outline and seemed to possess a distinct and comparatively resistant cuticle, while James states that they appear as if provided with a definite capsule. Bently also refers to a well-marked body-wall or resistant capsule.

Allusions have already been made to the opinion of some observers, who have considered the protozoa encountered in cases of splenomegaly, *kala-azar*, and *Oriental sore* as either the developmental forms of trypanosoma or those of a closely related species, or indeed as forms of sporozoa.

Ross, (46) in commenting upon Wright's discovery, mentions that a flagellated organism, *Cercomonas hominis*, is frequently found in superficial ulcers and in the intestines, and suggests that possibly these bodies of Wright may be forms of the same organism.

There can be little doubt as to the nature of the parasites encountered in my sections. They are, I believe, forms of *Blastomyces* (*torulæ*), though they are very different from the usual species of *Blastomyces* encountered in certain human skin affections. After a careful comparison of these bodies with those which have been found in ulcerations of the skin occurring in horses in the Tropics suffering from blastomycetic infection, I believe that the parasites of the two diseases are probably identical, and, as in glanders we have a disease which is occasionally transmitted to man, so human beings may also sometimes acquire this equine blastomycosis. However, it must be admitted that, when the parasites encountered in horses are compared side by side with those met with in the human case, slight differences may be observed. The equine organisms undoubtedly can be more clearly recognized as blastomycetic forms. They are also a little larger, their average length being about 5 μ, and their capsules frequently show a double contour, which has never been observed in the human parasite. However, these seem minor differences, and the similarity between the two is sufficiently great to make one feel that, even if the organisms are not identical, they must represent closely related species.

Cultures on agar were attempted from the lesion in my patient at the time the tissue was secured, but although large numbers of the parasites were inoculated, no apparent growth took place during several months. This is another point in favor of the identity of the two affections, since the parasites found in the horse are frequently very difficult or impossible to cultivate. Although the statement is often made in the literature that *Oriental boil* is communicable to the lower animals (Manson, Scheube, and Jeanselme), and that dogs, horses and rabbits have been inoculated with the discharges from the human lesions and successfully infected; nevertheless, it seems that further confirmation of this work is necessary. I did not at the time have the opportunity of inoculating a horse with any of the material from my case; but a monkey was injected subcutaneously with a portion of the fresh granulation tissue, shaken up in saline solution; though no pathological effect resulted. However, the monkey is also frequently immune against inoculation with the material from the form of equine tropical ulceration referred to. It is perhaps possible that had I not been familiar with the appearance of the torulæ encountered in this affection of horses, I might have mistaken these forms in the human lesion for protozoon-like bodies; since so much has recently been written of the occurrence of such organisms in *Oriental boil*, and since several observers state that the organisms encountered possess a definite capsule or cuticle.

SUMMARY.

My observations have led me to conclude that a number of forms of chronic ulceration of the skin are to be encountered in Manila, among which (after excluding certain ulcerative lesions of syphilis, yaws, leprosy, and lupus) there still exist at least several types of different etiology. A somewhat rare form is evidently of blastomycetic origin, in which the torulæ encountered have somewhat the appearance of the forms which have been described in certain cases of *Oriental boil* or *sore* as species of protozoa related to the Donovan-Leishman bodies.

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ILLUSTRATIONS.

FIGS. 1 TO 10. *Ulceration of the first type*[7] (*Delhi boil*).

Fig. 1. Section of the subcutaneous tissue, showing area of necrosis with extensive cellular infiltration and fibrin formation. In the fibrin mass just to the left of the center the parasites are very numerous.

2. A portion of the same field as fig. 1, more highly magnified. The parasites may be seen as small stained dots and oval bodies, some lying free but the majority inclosed in phagocytic cells.

3. Numerous parasites inclosed in endothelial phagocytic degenerating cells. The large phagocytic cell situated just above and to the left of the center of the field incloses a parasite showing the larger mass of chromatin and also the rod-shaped body.

4. Large phagocytic cell inclosing at least five parasites, in two of which the crescent-shaped, deeply staining mass of pigment is in focus.

5. Illustrates particularly two of the parasites not inclosed in cells, one lying almost in the center of the field and the other, a "seal-ring" form, lying below the center.

6. Single parasite with ring of chromatin to which is attached a short rod.

7. Free parasites and others inclosed in phagocytic cells.

8. Numerous parasites, many in clusters which show no stained chromatin mass in focus. The presence of considerable fibrin is evident.

9. Particularly showing single parasite in the center of the field.

10. Single parasite more highly magnified ("seal-ring" form).

11, 12. Leishman-Donovan bodies in a section from the spleen; photograph from one of Major Leishman's specimens; section sent through the kindness of Dr. Koch. The parasite in the center of each figure was selected to show the sharp outline of the limiting membrane.

13. Numerous Leishman-Donovan bodies photographed from the same specimen as figs. 11 and 12. In this photograph the stained chromatin masses are in better focus than the limiting membrane of the parasites, the latter here being indistinct, but in other portions of the section much more marked.

FIGS. 14 TO 18. *Ulceration of the second type* (*tropical sloughing phagadena*).

14. Gross lesion of ulceration of the second type, showing patches of pseudomembrane.

15. Section of tissue from the lesion pictured in fig. 14, showing area of infiltration, necrosis, and fibrin deposit.

16. Demonstrating area of coagulation necrosis and pseudomembrane. (Weigert stain).

17. Showing granulation tissue just below the necrotic area pictured in fig. 15 (more highly magnified).

18. Demonstrating proliferation of epithelioid cells about blood vessels.

[7] In this case the only photograph obtained of the gross lesion was one representing the scar; it is not reproduced, as it shows nothing characteristic.

FIGS. 19 TO 23.—*Ulceration of the third type (ulcerative dermatitis, Veld sore?).*

19, 20. Earlier and later gross lesions of ulceration of the third type.

21. Showing cellular proliferation about blood vessel (from unbroken nodule): the left side of the vessel wall is formed by the edge of the photograph.

22. Demonstrating character of cellular infiltration in the subcutaneous tissue (from unbroken nodule).

23. Section of tissue demonstrating early lesion, and region on the edge of an ulceration. On the extreme right of the photograph may be distinguished a small area of degeneration in the Malpighian layer of the epidermis: in the center, the infiltration of this stratum may be recognized; while at the extreme left, the partial erosion and necrosis of the epidermis is apparent.

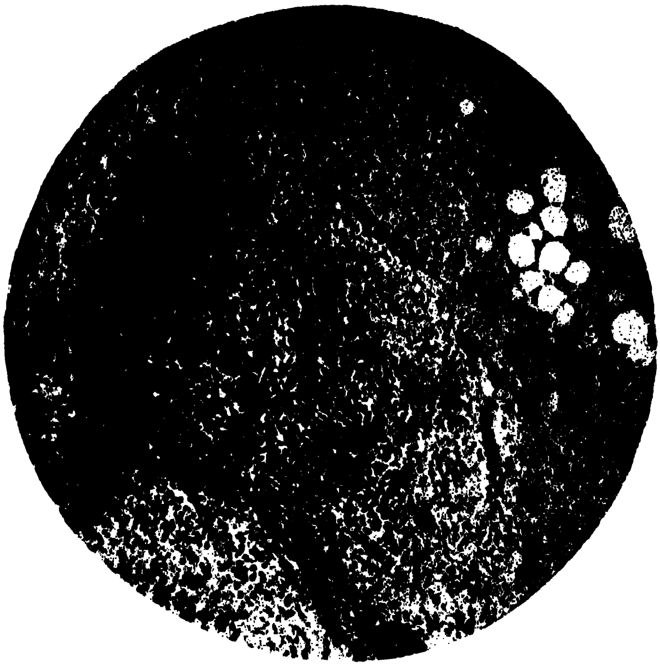

FIG. 1.

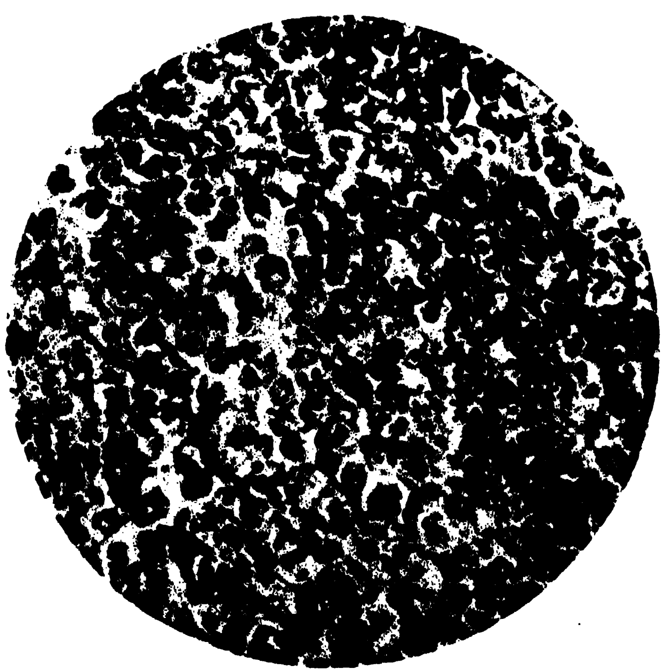

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

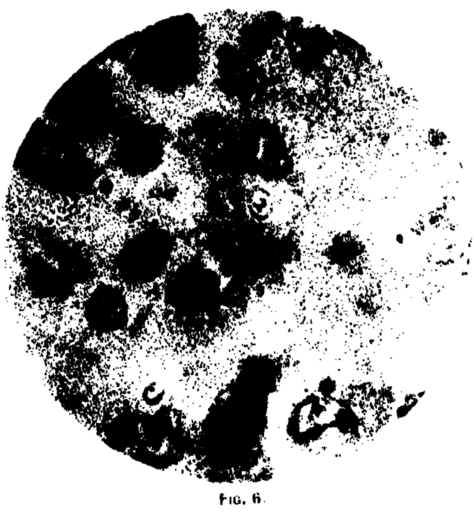

FIG. 6.

FIG. 7.

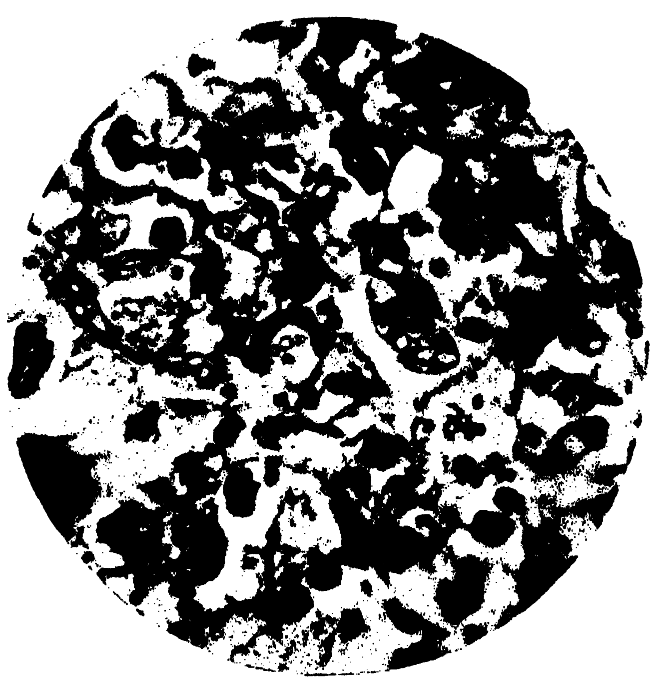

FIG. 8.

FIG. 9.

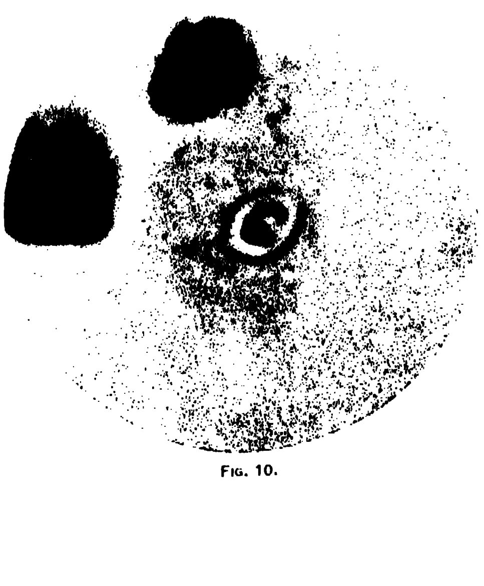

FIG. 10.

FIG. 11.

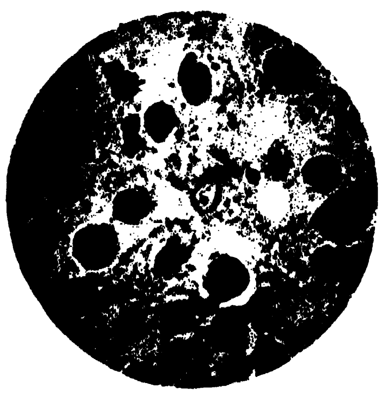

FIG. 12.

FIG. 13.

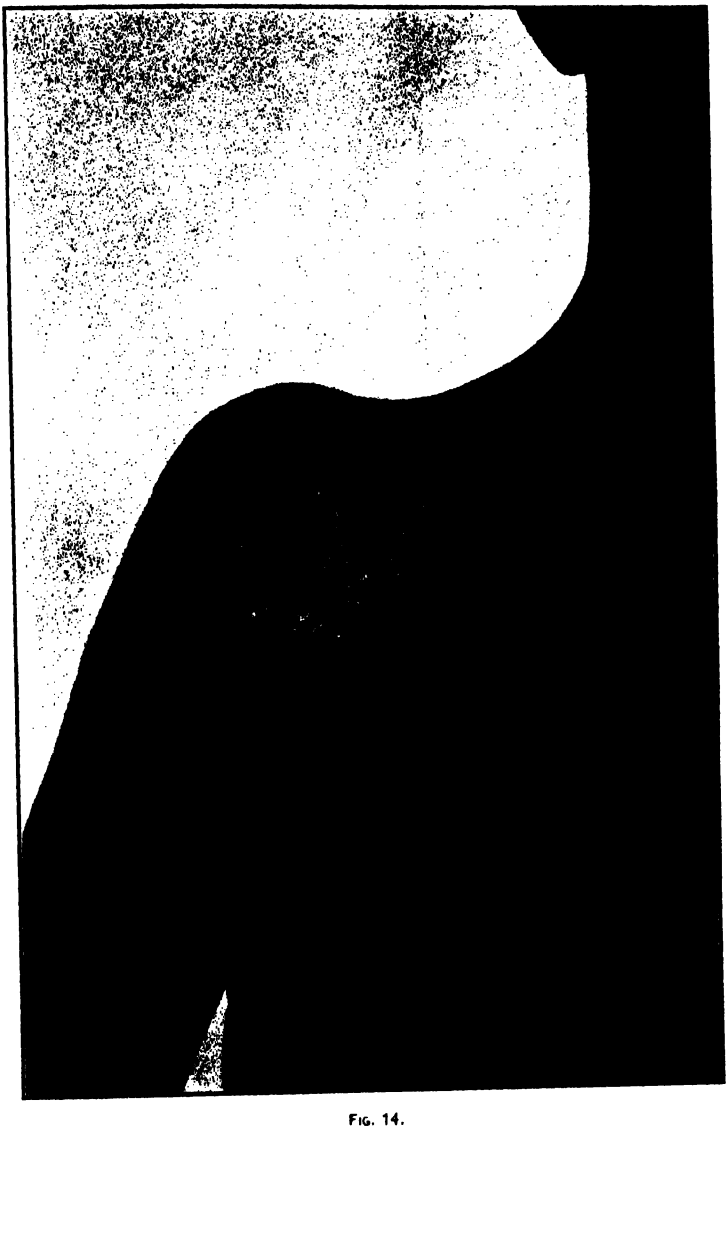

FIG. 14.

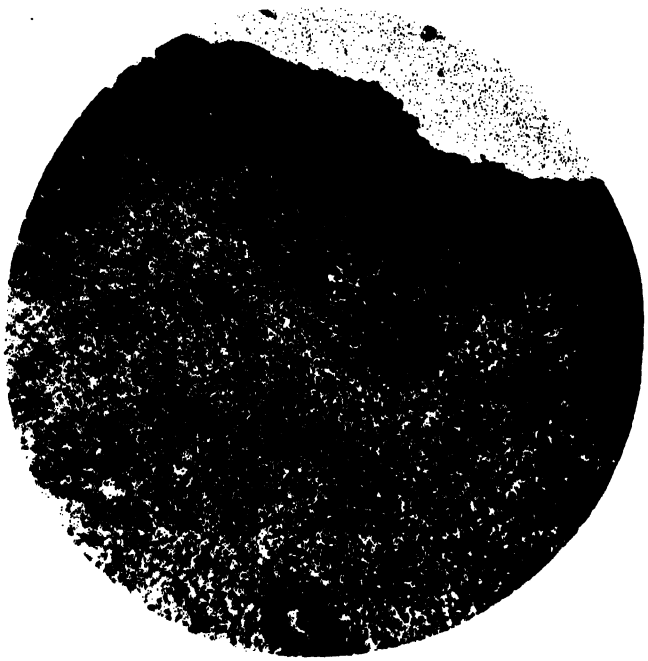

FIG. 15.

FIG. 16.

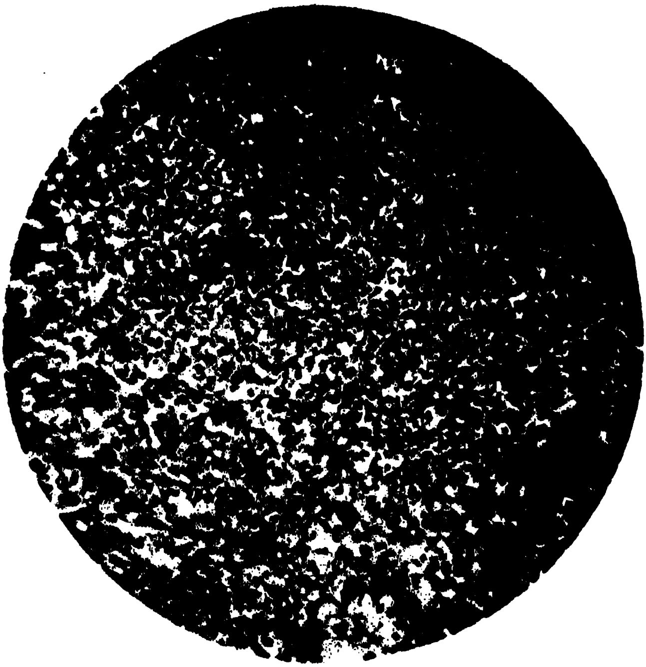

FIG. 17.

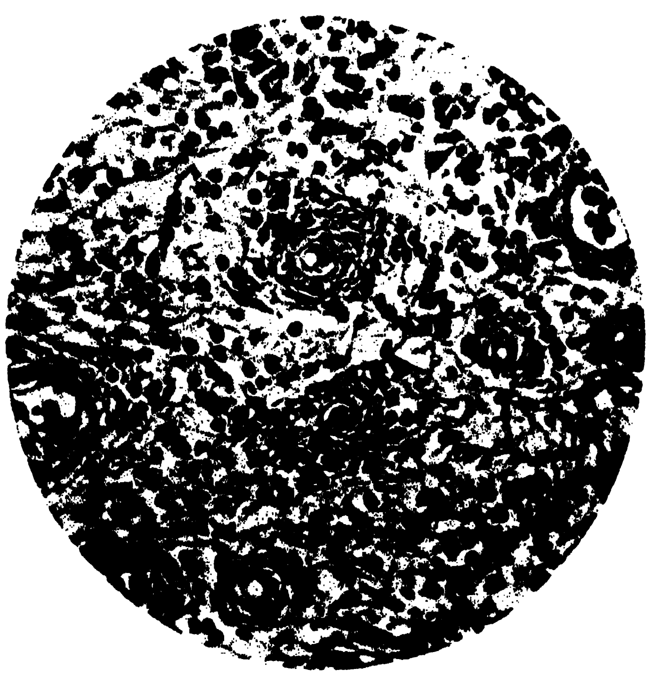

FIG. 18.

FIG. 19.

FIG. 20.

FIG. 21.

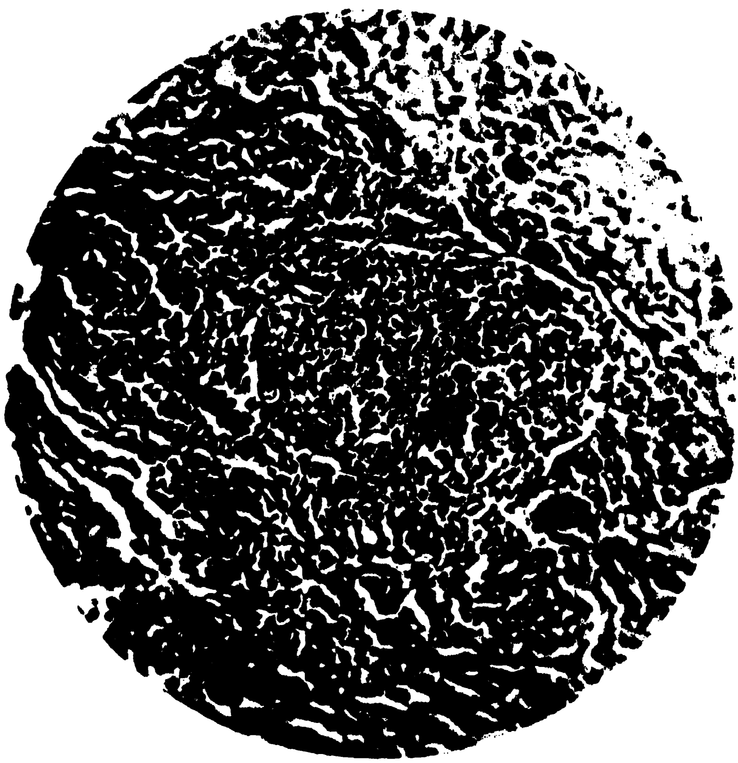

FIG. 22.

FIG. 23.

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THE KEEPING QUALITIES AND THE CAUSES OF RANCIDITY IN COCONUT OIL.

By HERBERT S. WALKER.

(*From the Chemical Laboratory, Bureau of Science.*)

In almost every work on fats and oils, coconut oil is cited as being especially prone to become rancid. Lewkowitsch[1] states that, when fresh, it possesses a bland, pleasant taste and odor, but that on standing it quickly becomes rancid. Samples analyzed by him contained from 5 to 25 per cent of free acid. Schestakoff[2] says that pure coconut oil shows an acid value (milligrams of caustic potash) of from 2 to 5. On standing under abnormal conditions, this may in one year rise to 60 or 70.

Coconut oil is in enormous demand as the basis of edible products such as "vegetable butter," etc., and therefore it is of the utmost importance to be able to produce an oil which, as nearly as possible, is free from fatty acids, rancid odor or taste, and which at the same time may be shipped without fear of deterioration.

The experiments to be described were undertaken with the view of discovering the conditions which induce a rapid deterioration of coconut oil, and, if possible, of ascertaining a means of improving its keeping qualities. In the course of this work it was noticed that the oil does not change with as great rapidity as is generally believed to be the case. The ordinary commercial oil, bought in Manila, contains from 5 to 10

[1] Lewkowitsch: *Chemical Analysis of Oils, Fats, and Waxes.*

[2] Schestakoff: Über den Gehalt an freien Fettsäuren natürlicher Fette und Öle. *Chem. Rev. Fett. u. Harz. Ind.* 9, 180.

per cent free acid, and there is no very great increase in acidity even on prolonged standing. Mr. Richmond, of this laboratory, in some experiments on a commercial oil, showed that it was not affected by passing a current either of dry or of moist air through the liquid for five days. An oil prepared by drying fresh coconut meat at 80° to 90° C., and then extracting with petroleum ether, was sterilized and sealed in a glass tube on August 16, 1904. At that time it contained 0.13 per cent free acid (calculated as oleïc). This sample was allowed to stand until February 21, 1905, and it was then again tested, with the result that it was in as good condition as at the time of the previous examination; there had been absolutely no increase in free acid. A sample of the same oil, kept in a sealed tin tube, on February 21, 1905, contained 0.34 per cent of free acid, an increase of only 0.21 per cent in six months. This same oil was allowed to remain in tin, unsealed, until April 11, 1905; the acidity had then increased to 0.46 per cent. On further standing in a glass-stoppered bottle until August 16, 1905, the figure was 0.50 per cent. An expressed oil from the same preparation of copra, kept under similar conditions, showed:

	Per cent.
February 21	0.77
April 11	0.78
August 16	0.96

This oil, from the start, had a slightly burnt odor and taste and in time it deposited a dark-brown sediment. However, neither of the two oils showed any signs of "rancidity" and even after a year had elapsed they were almost as pleasant to the taste as when first prepared.

In order more fully to study the effect of age and method of preparation on the keeping qualities of coconut oil, the following samples were prepared, their condition noted, and their exact titer determined. These oils will be allowed to stand for several years if necessary, until final results are obtained as to their respective rates of deterioration, but in the meanwhile the change up to the present time is given in the table which follows:

DESCRIPTION OF OILS USED AND DESCRIBED IN TABLE 1.

(A) Expressed oil from vacuum-dried copra. Has been heated for two hours at 100° and filtered twice through paper. A light-colored, clear oil with the characteristic coconut taste and odor.

(B) An oil similar in every respect to "A" except that it was prepared from copra dried at 80° to 90°, without vacuum.

(1) Fresh coconut meat grated and dried at 80° to 90° on August 16, 1904; was allowed to stand in a covered specimen jar until March 11, 1905. At that time it was still of a pleasant odor and taste, although both odor and taste were not quite as good as when the specimen was freshly prepared. No mold growth was present. A sample of oil was expressed from a portion of this copra by using a hydraulic press with a final pressure of 450 kilograms per square cen-

timeter. This oil, after filtration, was of a light-yellow color and it was of a pleasant, although slightly burnt, odor and taste.

(2) Oil No. 1 was heated at 100° for three hours, while at the same time a current of air in a partial vacuum was passed through it. This process leaves the color and free acid unchanged but removes almost all of the burnt odor, leaving a bland, almost tasteless, oil.

(3) An oil from the same copra as Nos. 1 and 2 but prepared by extraction with petroleum ether. Afterwards it was treated in the same manner as No. 2. It differs from Nos. 1 and 2 in being practically colorless.

(4) Commercial coconut oil treated with alcohol and animal charcoal and then filtered; the alcohol was afterwards distilled and recovered. This oil was rather unpleasant to the taste, but it had no odor.

(5) Commercial coconut oil treated with live steam; this removes the odor, but the unpleasant taste remains.

(6) Fresh meat, ground and dried in vacuum at 70° to 80°. The oil was expressed and once filtered; it possessed a very pleasant coconut-like odor and taste. It still contained a considerable amount of sediment.

(7) Coconuts cut in halves and dried in vacuum at 75° to 85°. The oil expressed and filtered twice. It had a very pleasant odor and taste.

(8) The same oil as No. 7, heated at 100° for one and one-half hours and filtered hot.

(9) The same as No. 7, heated at 100° for one and one-half hours, while at the same time a current of air was passed through the oil under partial vacuum. Filtered hot and bottled.

(10) Fresh coconut meat, ground and pressed in a hand press to remove most of the milk. Afterwards this meat was dried completely by spreading it in the sun for about five hours. The oil expressed from this copra was almost water white and without taste or odor.

(11) Coconuts split in halves and dried in the sun for five days. Ground and expressed. Yielded a cloudy, light-colored oil, very hard to filter, with a peculiar but not unpleasant taste and odor. This sample was strained through cloth but not filtered.

(12) Same as No. 11, strained and filtered slowly through paper.

(13) Same as No. 11, heated at 100° for two hours and filtered through paper.

(14) Fresh nuts, split in halves and allowed to stand during one week in the air at room temperature (about 30°). A vigorous mold growth and an unpleasant odor developed. This moldy meat was dried in a vacuum and the oil was expressed. This was highly colored and was rather unpleasant to taste and smell.

(15) Commercial coconut oil shaken with 2 per cent of solid calcium oxide (burned lime), heated to 100° and filtered. The filtrate was treated with animal charcoal and again filtered; there resulted a colorless oil which was very free from an unpleasant odor or taste.

(16) The same copra as that used for No. 1; was allowed to stand one month longer in an open jar, then expressed.

(17) Oil expressed from vacuum-dried copra which had stood for one month exposed to the air; the oil was heated to 100° and filtered.

(18) Expressed from sun-dried copra and treated in the same manner as No. 17. Both of these samples were of as pleasant a taste as oils from fresh copra.

(19) Vacuum-dried copra which had stood in a closed desiccator over water for one month, and which had accumulated a very decided growth of mold. It was dried for one hour and expressed. The oil had a considerable color and was slightly unpleasant as to taste and odor. Heated to 100° and filtered.

(20) Sun-dried copra treated in the same way as No. 19. Yielded an oil somewhat darker in color but otherwise much the same as No. 19. Filtered without heat.

(21) Same as No. 20, heated to 100° before filtering.

(22) The same copra as that used for samples 1 and 16 was allowed to stand for three weeks over water and for one week in air, and then dried and pressed. A vigorous mold growth appeared in the copra and a peculiar ethereal odor was apparent. The oil itself was of a light-yellow color, with a pungent, rather unpleasant, odor and an extremely disagreeable taste.

(23) Expressed from commercial copra, first quality, sun dried, Tacloban, Leyte. The unfiltered oil is dark colored and cloudy, depositing a black sediment.

(24) Same as No. 23, filtered. Almost colorless.

(25) Expressed from commercial copra, grill dried, Laguna (second quality). Not filtered.

(26) Same as No. 25, filtered. Light yellow in color.

(27) Expressed from commercial copra, grill dried, Romblon (considered second quality). The filtered oil is light yellow in color.

(28) Expressed from commercial copra, first quality, sun dried, Iloilo. The filtered oil is light yellow in color.[3]

(29) "*Langis*" coconut oil, prepared by the customary native process of grating the fresh meat, exhausting it repeatedly with water, and boiling down the emulsion thus obtained until it is nearly dry. The oil is then poured off from the brown coagulum which sinks to the bottom of the vessel. A freshly prepared oil, isolated in this manner, is very light in color and it possesses a decidedly pleasant coconut odor and taste. Before filtration it is more or less turbid, owing to the presence of a small amount of water and of albuminoids.

(30) Same as No. 29, filtered. The oil is water white.

(31) Best grade commercial coconut oil, probably made from fresh meat. It is light colored but very turbid and contains considerable water and suspended matter.

(32) Commercial coconut oil, probably made from copra. Very clear but highly colored.

(33) Commercial coconut oil, Manila. Probably made from fresh meat. It contained considerable suspended matter and water.

(34) Commercial coconut oil, Cebu. A highly colored "rancid" oil. Considerable sediment in the bottom of the bottle.

(35) Commercial coconut oil, Tayabas. A highly colored rancid oil made from copra. It is only a few months old.

The following table shows the change in the amount of free acid which has been produced in these oils while they were standing from the time of their expression up to the date of writing. The free acid was determined in each case by dissolving a known weight (about 5 grams) of oil in 50 cubic centimeters of neutralized absolute alcohol, and

[3] The copras from which these last six samples of oils were made were secured through the courtesy of Messrs. Smith, Bell & Co. and the Compañía General de Tabacos de Filipinas, and taken directly from their warehouses. The samples obtained were the ordinary grades of copra, ready for export, and had been stored for about two months, during the dry season. The oils, while not especially unpleasant to the taste, were of a sufficiently rancid character to preclude their use as edible products unless they were first subjected to a refining process.

then titrating with aqueous $\frac{N}{10}$ potassium hydroxide, using phenolphthaleïn as an indicator:

TABLE I.—*Percentage free fatty acids (as oleic).*

No.	At start.	Two months.	Four months.	Six months.
A[a]	0.06	0.06	0.09	0.60
B	0.06	0.06	0.08	[b]0.48
1	1.2	1.3	1.5	1.9
2	1.2	1.5	1.5	1.7
3	1.4	1.6	2.1	2.6
4	5.3		5.9	6.1
5	5.5			7.6
6	0.10	0.16	0.19	0.30
7	0.16	0.18	0.19	0.27
8	0.16	0.14	0.19	0.30
9	0.16	0.16	0.18	0.25
10	0.16	0.16	0.21	0.28
11	0.13	0.18	0.25	0.28
12	0.13	0.10	0.10	0.14
13	0.13	0.09	0.09	0.15
14	3.5	3.7	4.0	4.3
15	0.32		0.88	
16	1.6	1.7	2.0	
17	0.09	0.09	0.14	0.16
18	0.16	0.18	0.25	0.27
19	1.18	1.14	1.34	1.58
20	0.69	0.69	0.74	0.85
21	0.69	0.69	0.74	0.82
22	23.8			
23	1.4	1.6	1.8	2.0
24	1.4	1.5	1.7	1.8
25	2.6	3.4	3.6	3.9
26	2.6	2.6	3.1	3.5
27	2.1	2.4	2.5	2.8
28	3.0	3.5	4.0	4.7
29	0.08	0.38	0.60	0.69
30	0.08	0.13	0.16	0.19
31	2.0	2.9		
32	6.8	7.5	7.9	8.1
33	5.5		6.9	7.2
34	8.7		10.2	11.0
35	5.0	5.5		

[a]Through the courtesy of Prof. A. H. Gill, of the Massachusetts Institute of Technology, I have been given some further data on the keeping qualities of this oil. A sample packed in a sealed tin can was shipped to him at Boston and tested there when it was about three months old. On arrival it was described as being almost water white and of perfectly sweet odor. It then contained only 0.068 per cent free acid as oleic, a figure which corresponds very closely to that obtained here at the same time.

[b]This oil was kept in a large bottle. A sample in a small bottle showed an acidity of only 0.09 at this time.

At the present time there has been so little change in any of these samples that no very definite conclusions can be drawn as to the conditions which cause rancidity on standing. However, it may be considered as established that a pure, fresh, coconut oil can be prepared which contains a minimum amount of free acid and which shows no unpleasant

taste or odor. Such an oil very slowly increases in acidity, and, even after standing for one year under ordinary conditions, may still be edible without further purification.[4] Commercial oils, on the other hand, which contain from 5 to 10 per cent of free acid when freshly prepared, deteriorate much more rapidly, even though they have been filtered and are free from impurities. For example, No. 32, which, on first examination, had 6.8 per cent of free acid, increased in two months to 7.5 per cent. This oil is very clear, bright, and dry, being entirely free from sediment or turbidity of any kind. The samples prepared by us from fresh copra ranged from 0.06 to 0.16 per cent, the increase in two months being so small as to be almost negligible. Samples Nos. 1, 2, and 3, which contained a little over 1 per cent of free acid when fresh, increased from 0.1 to 0.3 per cent in the same time.

In fact, the increase in free acid to be expected in an oil when it is standing under ordinary conditions may almost be considered as being roughly proportional to its initial acidity. There are also indications that an oil from which albuminoids, etc., have been removed by filtration will retain its original condition better than one containing the above impurities. No. 6, for instance, which has been filtered once but which contained a considerable sediment, increased in four months from 0.10 per cent to 0.19 per cent, while Nos. 7, 8, and 9, oils prepared in a similar manner but filtered more thoroughly, only showed an increase in the same time of from 0.16 per cent to 0.19 per cent of free acid.[5] This fact was a little more noticeable among oils prepared from sun-dried copra. Samples Nos. 11, 12, and 13 were taken from the same lot of oil, the only difference being that No. 11 was left unfiltered, while the impurities were removed as completely as possible from Nos. 12 and 13. It will be noticed that No. 11, in six months, shows a total increase of 0.15 per cent free acid, having a little more than double its original acid value. Nos. 12 and 13 have in the same time increased only 0.01 and 0.02 per cent, respectively.

The oils prepared from commercial copra likewise show this distinction to a greater or less extent. No. 25, an unfiltered oil, increased from 2.6 to 3.4 per cent in two months, while No. 26, which is the same oil filtered, shows no change at all. However, contrary to expectations, the difference

[4] The sudden increase of acidity in samples "A" and "B" between the fourth and the sixth months is due to the abnormal conditions under which they were kept at this time. The two samples when originally prepared were kept in 500-cubic centimeter bottles which were nearly full, but during the fourth and fifth months they were opened so frequently for the purpose of taking samples for aldehyde and peroxide tests that only about 25 cubic centimeters remained in each bottle. The increase in acidity is probably due to a continuation of the surface oxidation which is discussed in a later part of this paper. A portion of sample "B" which had previously been removed to a smaller bottle showed practically no change at the end of six months.

[5] On further standing, however, the difference in this case is not so marked.

in behavior between a filtered and an unfiltered oil is most pronounced during the first few months. On longer standing, the acid values tend to approach each other.

The most important fact brought out by this work is that by far the greatest deterioration which an oil undergoes takes place in the copra itself. After an oil has been expressed from the dried meat, its change on standing is very slight compared with that which is found in the same time while it is in the copra. For instance, sample No. 1 was prepared from anhydrous copra, which had stood in a closed jar during about seven months (under much better conditions than copra is ordinarily kept); its free acid was 1.2 per cent; an oil expressed from this copra when it was fresh had only 0.77 per cent of free acid after it had stood for seven months in tin; this same copra after remaining three weeks over water and one week more in the air yielded an oil containing 23.3 per cent free oleic acid. Samples Nos. 19 and 20 were prepared from copra which, when fresh, gave an oil with almost no free acid. Fresh coconut meat on standing for even a short time in the air becomes covered with mold and produces an oil of a more or less rancid character (cf. No. 14). No great amount of rancidity was developed in any case until signs of mold or bacterial growth were visible on the surface of the copra. From this it would seem very probable that the splitting up of fat and the accompanying "rancidity" produced in copra are in a large measure due to the action of micro-organisms, which have an excellent culture medium in the sugar, albuminoids, and water which exist, together with the oil, in coconut meat.

Koenig, Spiechermann, and Bremer,[a] in their valuable paper on the decomposition of fats by micro-organisms, have conclusively shown that cottonseed meal containing a sufficient amount of water, is attacked by molds and bacteria, and that the oil therein is, on long standing, almost completely destroyed. In the accompanying experiments the methods used by these authors were followed, with certain modifications, which consisted chiefly in substituting freshly prepared anhydrous copra for cottonseed meal, and in paying especial attention to the amount of free acid developed.

The copra used for this work was prepared by grinding up fresh coconut meat and drying it at 90° to 100° C. under a partial vacuum, until the meat was anhydrous. It was then kept over sulphuric acid, to be used as needed. This product had become quite brown during the prolonged drying, but yielded an almost colorless oil, of a sweet taste, and which contained about 0.15 per cent free acid as oleic.

Ten-gram samples were weighed out in large, stoppered test tubes and each tube was inoculated with one drop of a solution made from some

[a] Koenig, Spiechermann, und Bremer: Beiträge zur Zersetzung der Futter- und Nahrungsmittel durch Kleinwesen. I. Die Fettverzehrenden Kleinwesen. *Ztschr. f. untersuch. d. Nahrungs-u. Genussmittel* (1901), 4, 721, 769.

old, moldy copra. A definite volume of distilled water was then added to each, and all were allowed to stand for one week at room temperature (25°–30° C.) and for the same length of time in an incubator at about 35° C., the tubes being opened every morning and any change in odor or appearance noted. After two weeks the whole series was dried, weighed, the oil extracted with chloroform, and its acidity determined.

	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	No. 9.
Weight of dry copra	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Weight of water added	0.00	0.50	1.00	1.50	2.00	3.00	5.00	7.00	10.00
Percentage moisture	0.00	4.76	9.09	13.04	16.67	23.08	33.33	41.18	50.00
Weight of dry copra at end of experiment	10.00	9.99	9.70	9.20	9.30	9.10	9.20	8.97	9.15
Gain (+) or loss (—)	0.00	−0.01	−0.30	−0.80	−0.70	−0.90	−0.80	−1.03	−0.85
Weight of oil	6.79	6.77	6.61	6.64	6.68	6.82	6.78	6.67	6.84
Gain (+) or loss (—)	0.00	−0.02	−0.18	−0.15	−0.11	(+0.03)	−0.01	−0.12	(+0.05)
Gain (+) or loss (—) of substances other than oil	0.00	(+0.01)	−0.12	−0.65	−0.59	−0.93	−0.79	−0.91	−0.90
Percentage free acid	0.15	0.18	8.7	5.2	3.9	0.46	3.0	6.1	2.9

Nos. 1 and 2 remained unchanged in appearance and odor throughout the experiment. Nos. 3, 4, and 5 developed a slight mold growth and a peculiar ethereal odor, not especially unpleasant except in the case of No. 5. No. 6 showed no mold growth but turned much darker than the others and almost from the start possessed a very disagreeable odor. Nos. 7, 8, and 9 soon developed a sour smell, which, however, was not so unpleasant as that of No. 6, but they showed no mold until they had been placed in the incubator, when Nos. 7 and 8 became covered with a vigorous white growth with numerous patches of red.[7] No. 9 remained unchanged in appearance.

The percentage of free acid in the oil here seems very closely to follow the appearance of a visible mold growth in the copra, being at a maximum in No. 3, where the mold first makes its appearance, decreasing slowly, with added moisture, up to No. 6 (no mold growth in evidence), increasing again in Nos. 7 and 8 (reappearance of mold growth).

As might be expected in an experiment of so short a duration, the loss in total weight of oil was in no case large, but it was sufficiently marked to show that it also chiefly took place in those tubes which contained a

[7] Subsequent experiments indicate that this growth in Nos. 7 and 8 was due to a loss of moisture while in the incubator.

growth of mold; the loss of substances other than oil, on the contrary, was considerably less where the mold was most vigorous.

As nothing was known concerning the organisms with which these tubes had been inoculated, it was decided to repeat this experiment, without inoculating the tubes directly, but to start the growth by simply exposing them to the action of such organisms as might be present in the air from day to day. Therefore, the tubes were filled as before and allowed to stand at room temperature for two weeks, being opened and exposed to the air for a few minutes each day. A similar set was prepared at the same time and afterwards given to Dr. Edwards, of this Bureau, for bacteriological examination.

	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	No. 9.
Weight of dry copra	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Weight of water added	0.00	0.50	1.00	1.50	2.00	3.00	5.00	7.00	10.00
Percentage moisture	0.00	4.76	9.09	13.04	16.67	23.08	33.33	41.18	50.00
Weight of dry copra at end of experiment	9.99	10.00	9.67	9.59	9.39	9.11	9.42	8.97	(*)
Gain (+) or loss (−)	(−0.01)	0.00	−0.33	−0.41	−0.61	0.89	−1.58	−1.03	
Weight of oil	6.83	6.85	6.24	6.18	6.54	6.92	6.81	6.83	
Gain (+) or loss (−)	0.00	(+0.02)	−0.59	−0.65	−0.29	(+0.09)	0.02	0.00	
Gain (+) or loss (−) of substances other than oil	(−0.01)	(+0.02)	+0.26	+0.24	−0.32	−0.98	−0.56	−1.03	
Percentage free acid	0.15	0.17	11.8	12.9	13.7	0.57	0.39	0.47	0.24

*This tube was broken while drying.

These tubes behaved very much like those which had been used in the previous experiment, Nos. 4 and 5 showing a growth of mold and an ethereal odor in four and No. 3 in six days; No. 6 darkened and became putrid, while Nos. 7, 8, and 9 simply turned "sour."

The bacteriological examination showed no organisms to be growing in Nos. 1 and 2; bacteria were found in Nos. 3 to 9, inclusive, and molds in Nos. 3, 4, and 5 only; the latter were much more numerous than the bacteria in Nos. 3 and 4, and about equally divided in No. 5.

The mold most commonly occurring in Nos. 3, 4, and 5 was identified as *Aspergillus flavus;* others, mostly *Aspergilli,* were also found but as yet have not been indentified. Quite a number of bacilli were isolated in pure cultures, but no attempt at identification has yet been made.[8]

[8] Experiments are now being undertaken to study the action of pure cultures of all these organisms on copra of varying degrees of moisture. The cultures are being prepared by Dr. Edwards. The results will be published in a later paper.

In this series of tubes, as well as in the preceding one, a high acid value, accompanied by a loss in weight of oil, is evident only in those samples which have been attacked by molds—that is, in Nos. 3, 4, and 5, which had a water content of from 9.09 to 16.67 per cent.

No. 6, the dark-colored sample with a very disagreeable odor, showed a slight gain in the weight of oil, probably due to the production, by organisms, of bodies other than oil which are soluble in chloroform. In all the other samples the weight of the oil practically remained unchanged. The large loss in substances other than oil (sugars, albuminoids, etc.) is confined, on the contrary, to those tubes in which bacteria predominate—that is, those containing more than 16.67 per cent of moisture—indicating that bacteria obtain their carbon and hydrogen chiefly from the sugars, albuminoids, and cellulose which are present in copra, while molds directly attack the oil. Whether molds alone can split up and assimilate oil from copra, or whether they may not be symbiotic with certain bacteria, remains to be established by means of the experiments to be undertaken with pure cultures.

The most important point to be considered from a practical point of view is the fact that copra containing as little as 9 per cent of moisture is still attacked by molds, with the consequent production of free acid and coloring matter as well as loss in weight of oil. Unfortunately, the copra produced in the Philippine Islands ordinarily contains from 9 to 12 per cent of water, a condition which is the most favorable for mold growth and for the deterioration of the oil. The remedy for this is obvious. A more complete drying, to reduce the water content to 5 per cent or less, will produce a copra which is unattacked by organisms. Such a product, kept dry, will remain fresh and sweet for a long time. In a previous part of this paper I have shown that copra, once sufficiently dried, may be kept during the dry season in Manila without any change whatsoever, but recent experiments prove this not to be the case during the rainy one, even with anhydrous copra.

Two samples of the latter, cut into fine pieces, were exposed, in open specimen jars, for a period of one month. At the end of this time one sample was covered to exclude air, while the other remained open. The covered sample soon developed a slight mold growth and a characteristic ethereal odor, and at the end of another month the oil extracted from it contained free acid to the amount of 3 per cent. The sample left uncovered for two months was not changed as much, for the oil from it contained only 0.89 free acid. This is probably due to the fact that during the time of exposure there occurred several comparatively dry periods of from three to four days each, during which there was very little rain, thus giving the specimen an opportunity to become partially dry so that the beginning growth of any mold would be stopped. The covered and uncovered samples were found to contain 7.8 and 6 per cent of moisture, respectively, which indicates the marked influence of a

comparatively small amount of water on the keeping qualities of copra. As shown in the previous experiment, copra containing 4.76 per cent of moisture remains practically unchanged on standing under conditions which preclude the absorption of water, while that with 9.09 per cent produced 11.8 per cent free acid in two weeks. Between these two extremes come the two samples mentioned above, the one with 6 per cent of water increasing to 0.89 per cent and that with 7.8 per cent rising to 3 per cent of free acid during a period of two months.

EXPERIMENTS ON COPRA DRYING.

Since the quick and thorough drying of copra has been shown to be of such vital importance in order to insure the production of a pure oil, an investigation of various methods of copra drying has been made, taking into consideration not only the processes common in these Islands but also those which are used in other countries.

Sun drying.—As has been stated in the introduction to this series of papers, the simplest and most primitive mode of drying copra is to expose the nuts, cut in halves, to the action of the sun during about five days. This method, although it is a slow one, under favorable climatic conditions produces a very fair quality of copra. However, a sudden rainstorm or a succession of cloudy days is sufficient to start mold and bacterial growth, with the consequent deterioration of the copra. Considerable loss due to the attacks of insects and animals is also suffered during the long period of drying, and the finished product very seldom contains less than 9 per cent of moisture.

Grill drying.—A much quicker method is the one carried out by laying the half nuts, face downward, on a bamboo grating placed over a slow fire of coconut husks. After being dried in this manner over night the nuts are removed from their shells and are then again placed over the fire, where they are allowed to remain for from four to five hours longer. This process, although it is cheap and comparatively rapid, has the disadvantage of yielding a dark-colored product which has a smoke-like taste and odor, and it also tends to form a hard, burnt coating over the surface of the nut while the inside is left in a comparatively moist state, a fact which is often taken advantage of by the small producers, who sell their copra by weight. Commercial copra prepared in this way contains from 9 to 13 per cent of moisture.

Hot-air drying.—This method of desiccation has been used successfully for a long time in the preparation of coffee, cacao, dried fruits, etc., and is at present in quite extensive use for the making of copra in Ceylon,[a] where it is said to give a very pure, light-colored product. The type of apparatus used in that island essentially consists of a large chamber filled with wire trays upon which the coconuts are placed and over which a

[a] *Tropical Agriculturalist*, 23, No. 10, supplement.

current of hot air, driven by a fan, is passed. In Trinidad,[10] British West Indies, there is now in operation a rotary hot-air drier which, it is stated, is better than any other apparatus now in use.

For the purpose of testing the efficiency of the stationary form of hot-air drier, a double-walled, rectangular galvanized-iron box, having an internal capacity of about 0.2 cubic meter, was constructed. Three galvanized-iron trays, perforated at one end, were set in this box in such a manner that the stream of hot air entering through a 20-centimeter pipe at the bottom was compelled to pass over each in turn before escaping at the top of the apparatus. A constant current of air was obtained by means of a small electric fan which was connected with a section of 15-centimeter pipe, so arranged that it could be heated by a small kerosene stove to any desired temperature. The apparatus had a maximum capacity of 24 nuts split in halves or 12 nuts when shredded.

Experiment I.—Four nuts were split in halves and placed on the bottom tray.

Temperature of entering air, 56° C.
Temperature of escaping air, 51° C.
Time of drying, 20 hours.

The copra dried at this comparatively low temperature was very white and of the best quality. A sample of oil expressed from it contained 0.08 per cent free acid.

Experiment II.—The meat from twelve nuts was shredded by hand and treated for one day in the same manner as in the preceding experiment; it was then allowed to stand at room temperature over night and completely dried on the following day. The substance in the bottom tray naturally desiccated much more rapidly than in the other two, therefore as soon as one tray was completely dry it was removed and replaced by the one just above it.

Temperature of entering air, 56° C.
Temperature of escaping air, 50° C.
Actual time of drying:
Top, 14½ hours.
Middle, 12¼ hours.
Bottom, 9½ hours.

The less completely dried copra in the two upper trays became slightly "soured" while standing over night. This caused a slight increase in free acid as follows:

Tray.	Per cent free fatty acid.
Top	0.32
Middle	0.16
Bottom	0.13

[10] *Journal d'Agriculture Tropicale* (1904), 103.

Experiment III.—The meat from four nuts was shredded and placed in the bottom tray, being stirred every half hour.

> Temperature of entering air, 93° C.
> Temperature of escaping air, 74° C.
> Time of drying, 3½ hours.

The copra thus produced was thoroughly dry, very white, and pleasant to the taste. The oil expressed from it contained only 0.06 per cent free fatty acid.

In Experiment II, as would naturally be expected, it is evident that the meat farthest away from the entering air requires a much longer time for drying than does that which lies closer to the bottom of the box. This is due to the fact that the air gradually becomes cooler and more completely saturated with water vapor as it passes over the moist copra. For practical use, therefore, a drier should be equipped with some sort of a mechanical carrier which would constantly introduce fresh coconut meat at the coolest part of the machine and then bring it slowly down toward the hottest portion.

Experiment IV.—This was undertaken in an endeavor to ascertain the approximate time required completely to dry the fresh meat, introducing it at the top of the apparatus and shifting it gradually toward the bottom. Four trays, each containing the freshly grated meat of 4 coconuts, were prepared, and three of these were placed in the drier simultaneously, tray No. 1 being at the bottom. After the latter had become sufficiently dry, it was removed from the apparatus and tray No. 2 moved down to take its place; this was next replaced by No. 3, and finally in the same manner by the moist sample No. 4.

> Entering air, 95° C.
> Escaping air, 70° C.
> Actual time of drying:
> No. 1, 4¼ hours.
> No. 2, 5¼ hours.
> No. 3, 6¾ hours.
> No. 4, 4 hours.

From the above experiments it may be concluded that the average time of drying, where the apparatus is run continuously at 95° C., will approximately be four hours.

The rotary drier.—A section of galvanized-iron pipe 20 centimeters in diameter by 6 meters long was set up on wheels and connected with a small electric motor so that it could be made to revolve at any desired speed. The same current of hot air which was previously used for the stationary drier was connected with this apparatus. Four strips of angle iron extending throughout the length of the pipe served to keep the moist copra in constant motion during the time of drying. After much preliminary work to determine the proper inclination necessary to allow the material to pass through the apparatus with sufficient slowness, it was

found that by careful manipulation the grated meat from four nuts could be dried in about two hours so as not to contain more than 6 per cent of moisture. The only objection to this method consists in the difficulty of regulating the speed with which the ground meat passes from one end of the apparatus to the other. This is dependent on four factors: (1) The number of revolutions per minute, (2) the angle of inclination, (3) the specific gravity of the coconut meat, and (4) the speed of the entering current of hot air. In the machine used here an unfortunate tendency toward a separation of the moist from the dry copra appeared; the dry particles, being lighter, were held back by the current of air or even blown out through the upper end of the tube, whereas the moist and consequently heavier pieces passed through too quickly. When these mechanical difficulties are solved this should prove the ideal method for drying coconut meat for oil-making purposes.

Vacuum drying.—The apparatus used was a small, barrel-shaped iron chamber, about 34 centimeters in diameter and in length, insulated with asbestos and heated by three hollow steam plates upon which the substance to be dried was placed. The pump connected with this drier gave a vacuum of about 660 millimeters (absolute pressure of 100 millimeters).

Experiment I.—Four coconuts (the maximum capacity of the apparatus) were split in halves, after removing the outer husk, and kept in the drier for three hours. The meat had then contracted sufficiently to allow of its being removed from the shell. During this time the temperature had gradually risen from 30° to 80°. The meat was then subjected to a further drying during four hours, at the end of which time, though not perfectly anhydrous, it was fully as dry as the ordinary commercial article.

Actual time of drying, 7 hours.
Maximum temperature, 80° C.
Vacuum, 635 millimeters.
Steam pressure, about 0.7 kilo per square centimeter (10 pounds).

Experiment II.—The preceding experiment was repeated under practically the same conditions, except that the nuts were allowed to dry completely without removing the shell.

Time of actual drying, 8 hours.
Maximum temperature, 80° C.
Vacuum, 648 millimeters.
Steam pressure, about 0.7 kilo per square centimeter (10 pounds).

Experiment III.—An attempt was made to shorten the time of drying by increasing the steam pressure and having the machine hot before putting in the nuts, the initial temperature being 75°.

Actual time of drying, 5¾ hours.
Maximum temperature, 85° C.
Vacuum, 640 millimeters.
Steam pressure, about 4.2 kilos per square centimeter (60 pounds).

Experiment IV.—The meat from four nuts was ground and spread in a layer of a depth of about 3 centimeters in shallow glass dishes. The initial temperature was 60°.

Actual time of drying, 9 hours.
Maximum temperature, 75° C.
Vacuum, 660 millimeters.
Steam pressure, 0.7 kilo per square centimeter (about 10 pounds).

Experiment V.—Four coconuts were split in halves and put into the machine, the latter being left partly open and with no vacuum.

Actual time of drying, 11 hours.
Highest temperature, 86° C.
Steam pressure, 0.7 kilo per square centimeter (about 10 pounds).

Therefore, under the best conditions obtainable (temperature 85° and vacuum 635 to 660 millimeters), the minimum time required for vacuum drying was five and one-half hours.[11]

If we are to form our judgment from the great efficiency of the vacuum evaporators used for sugar solutions and for many other liquids, it might be supposed that this process would be equally advantageous for coconuts. However, the two conditions are altogether different. In the case of solutions we have a thin layer of liquid in direct contact with a heated surface, the evaporation taking place so rapidly that the space above the liquid is constantly saturated with moisture; the main object of these machines is to remove and condense the surplus water vapor as rapidly as possible and by so doing to allow the evaporation to proceed at a comparatively low temperature. The water in coconut meat, on the other hand, which at the most is not greater than 50 per cent of the total weight of material, under the best conditions, diffuses very slowly through the cells of the copra to the surface, the removal of moisture-laden air therefore becoming a matter of secondary importance. The principal consideration is the constant application of as much heat to the entire surface of the material as the latter can endure without becoming burnt. That this condition is not fulfilled in the best manner by a vacuum drier is chiefly due to the poor conductivity of the rarified air which it is necessary to heat. Although the temperature of the steam plates in the drying oven is from 100° to 110°, that of the partial vacuum immediately above and surrounding the copra, even after several hours, rarely rises above 75°. To this local superheating for a long period of time at the point of contact with the plates, probably is due the brown color and slightly burnt taste which vacuum-dried copra almost invariably possesses.

For the sake of comparison I append the following table showing the

[11] This does not include the time necessary to produce steam and to heat up the drier. These items must be considered unless the apparatus is to run continuously.

approximate time required to dry copra under the most favorable conditions by each of the methods previously considered:

Method.	Time.
Sun	5 days.
Grill	10 to 12 hours.
Hot air (box)	3½ to 4 hours.
Hot air (rotary)	2 to 3 hours.
Vacuum	5½ to 6½ hours.

The quality of the copra produced by the hot-air box drier is very much superior to that yielded by any other method, since it is perfectly white and dry, retaining the pleasant odor and taste of fresh coconut meat. For oil-making purposes the rotary apparatus, because it lends itself to a continuous process and requires considerably less time, recommends itself especially, although its product does not present quite so pleasing an appearance. Either of these two methods, on account of their cheapness and simplicity, should be preferred to vacuum drying.

Centrifugating.—Another method of drying suggests itself, which should prove to be very efficient, although, owing to lack of facilities, I have not as yet been able to give it a practical test. This is to extract the meat from coconuts by means of a rotary burr and to run this product directly into a powerful centrifugal from which the greater part of the water would be thrown off at once. A comparatively short, supplementary drying by means of hot air would then suffice to prepare copra for expressing the oil. Another point in favor of this method is that the copra resulting therefrom, having lost most of its sugar and albuminoids together with its water in the process of centrifugation, would be able to withstand a higher temperature while drying (with a resulting economy of time) without showing the same tendency to turn brown. Once dry, it could be stored with less danger of deterioration through mold action than material prepared by ordinary methods. The objection may be raised that, during the centrifugation, a considerable amount of oil together with the water would be thrown off from the fresh meat, and that this would either entirely be lost or would necessitate much labor for its recovery. This, to a certain extent, is true, as the water in coconut meat exists in the form of a cream-like emulsion with oil, sugar, and albuminoids. A sample of this "coconut cream," prepared by expressing the fresh meat in a hand press, was, on analysis, found to have a specific gravity of 1.012 at 30° C. and to consist of—

	Per cent.
Water	56.3
Total solids	43.7
Ash	1.2
Fat	33.4
Proteid (N x 6.25)[12]	4.1
Total sugar as invert sugar	5.0

[12] Determination made by Mr. Richmond, of this laboratory.

The above results show that it approximates in nutritive properties the composition of a rich, natural cream; it is very pleasant and sweet to the taste, possesses an agreeable odor, and, when sterilized and properly sealed, will remain indefinitely in a fresh condition. Such a product could be used as as substitute for all of the purposes to which the so-called "evaporated creams," now on the market, are put, and it might prove to be one of the most valuable by-products of the coconut-oil industry.

THE ACTION OF ORGANISMS ON COCONUT OIL UNDER VARYING CONDITIONS.

Although, as has been shown above, the character of a coconut oil in regard to free acid, odor, and taste is determined chiefly by the quality of the copra used for its production, there is also in most commercial oils a slow but steady deterioration, amounting in the worst cases to a rise of about 0.5 per cent per month (cf. sample No. 31, p. 120), while with pure, filtered oils this reaches only a few hundredths per cent in the same time. It has been remarked above that samples of oil which contain suspended impurities and water, as a rule, increase in their content of free acid somewhat more rapidly than do similar ones which have been clarified; a result to be expected, if, as is the case with copra, decomposition is due to micro-organisms, since it has been proven that bacteria and molds do not live for any length of time in pure oil.[13]

The influence of impurities on the keeping qualities of oils was noticed as early as 1855 by Pelouze,[14] who observed that various oleaginous seeds, when crushed and extracted at once, yielded almost neutral oils, whereas if, after being crushed, they were allowed to stand for some time before extraction, the oil then produced contained a large amount of free acid. He considered this action to be due to a "ferment" similar to that producing alcohol from sugar.

Pastrovich and Ulzer,[15] using a mixture of oleomargarine with 0.5 per cent casein and 1 per cent water, observed an increase of acidity from 0.888 to 1.250 per cent in one week, and in fourteen weeks 0.888 to 10.270 per cent. They make no attempt to explain this effect, evidently attributing the saponification to some change brought about directly by the presence of albuminoids, although it is very probable that it was produced by bacteria or molds.

The following experiments were undertaken with a view of accentuating this difference in keeping qualities between pure and impure oils by exposing them directly to the action of micro-organisms under similar conditions.

About 20 cubic centimeters each of samples Nos. 6, 8, and 11 were poured into small beakers and placed in a covered specimen jar containing

[13] E. Ritsert: Untersuchungen über das Ranzigwerden der Fette. *Chem. Centrb.* (1890), 507, 575, 813.

[14] M. J. Pelouze: Memoir, sur la saponification des huiles sous l'influence des matieres qui les accompagnent dans les grains. *Compt. Rend.* (1855), 40; 605.

[15] Pastrovich u. Ulzer: Ueber den Einfluss der Gegenwart verschiedener Eiweisskörper auf Fette. *Ber. d. chem. Gesell.* (1903), 36, 209.

a little moldy copra together with a beaker of water to insure an abundance of moisture. They were then allowed to stand at room temperature (25° to 30° C.) for two months. Their change in acid value is shown as follows:

Number of oil.	After standing one month.		After standing two months.	
	F. F. A. in oil exposed to mold.	F. F. A. in oil in original bottles.	F. F. A. in oil exposed to mold.	F. F. A. in oil in original bottles.
6	0.22	0.16	0.26	0.17
8	0.23	0.14	0.26	0.17
11	0.22	0.18	0.83	0.22

A slight increase of acidity was evident in each of these samples, but No. 11, an unfiltered, very turbid oil, was decomposed much more rapidly than either of the others. It also was characteristically rancid in odor and taste and contained a visible mold growth.

Since a marked difference was shown in the behavior of these oils, it was decided to observe the effect of the addition of small quantities of nutrient matter and of water on the rise in the free-acid contents of a pure oil. The nutrient material which was used was prepared from "latic," a coagulated residue produced in the native process of making oil, by boiling down an emulsion of fresh coconut meat. This residue, when dried and extracted with chloroform, yields a light-brown powder, partially soluble in water and of a sweetish, not unpleasant, taste. It consists chiefly of albumin and sugar. The following samples were prepared, using pure, fresh oil as a base:

A. 25 cubic centimeters oil + 0.25 grams "latic."
B. 25 cubic centimeters oil + 0.25 grams "latic" + 0.25 cubic centimeters water.
C. Control of pure oil.

Each of these samples was placed in a 50-cubic centimeter glass bottle inoculated with one drop of the moldy oil (No. 11) used in the previous experiments, and allowed to stand for one week at room temperature, and, after determining the increase in acidity, for one week in the incubator at 35°. Finally, the acidity was tested after the oil was allowed to stand for two months longer at room temperature.

Sample.	Initial acidity.	One week at room temperature.	One week at 35° C.	Two months at room temperature.
A	0.10	0.11	0.13	0.27
B	0.10	0.25	0.60	3.8
C	0.10	0.10	0.11	0.13

The following table gives the results of a similar experiment in which 10 cubic centimeters of the oil to be tested were inoculated with a drop of moldy oil, then poured out in a Petrie dish, which was placed in a closed specimen jar over a little moist, moldy copra, and allowed to stand in the incubator at 33° to 35° C. for one week:

Nature of oil.	Initial acidity.	One week at 33°-35° C.
No. 29	0.10	0.50
No. 30	0.10	0.21
No. 30 + 1 per cent "latic" + 1 per cent water	0.10	8.63

The last sample, which showed the greatest increase in acidity I have yet been able to produce in a short time, was almost completely covered by a greenish-yellow mold, similar to that noticed on copra, and it had the characteristic odor of a rancid oil.

In the light of these latter experiments there can be no doubt of the fact that coconut oil, provided it contains sufficient moisture and nutrient matter, is attacked by micro-organisms, principally molds, with an accompanying production of free acid and of a disagreeable taste and odor. This is the principal cause of "rancidity" in coconut oil, if by "rancidity" we mean a high acid content and a bad taste and odor. Whether this fat splitting is directly due to a life process of the molds or to an enzyme secreted by them is a problem which has not yet been solved. However, it seems highly probable that these molds produce a slowly acting enzyme, soluble in oil, which continues its hydrolytic action even after the organisms themselves are dead. This would account for the steady increase in free acid of some commercial oils which are perfectly clear and free from impurities and which have been proven to contain no living bacteria or molds. Experiments are now being carried on to clear up this point.

OTHER FACTORS INFLUENCING THE ACIDITY OF OIL.

Effect of sunlight.—Twenty-five cubic centimeters each of samples "A" and "11" (see table, p. 119) were placed in 50-cubic centimeter glass-stoppered bottles and allowed to stand in the sun for one month. At the end of this time "A" contained 0.22 per cent and "11" 0.24 per cent of free acid, while at the same time the original samples "A" and "11" showed 0.06 and 0.18 per cent, respectively. No marked change in taste or odor could be detected. The acid content of the pure sample "A" appears to have increased considerably more than that of "11," due probably to its contamination with a few drops of water during a heavy rain. However, the total amount of acid developed was so small that the experiment was not repeated.

Effective of heat and moisture.—Several samples of oil were heated at 100° for periods up to twenty-four hours with no change in acid

value. However, on heating in a sealed tube, considerable alteration took place, as is shown in the following: Samples from No. "B" and from that portion of No. 11 which had been exposed to sunlight were heated in sealed tubes at about 160° for ten hours. "B" changed in percentage of free acid from 0.06 to 0.90, but was unchanged in color, odor, or taste. "11" rose from 0.24 per cent to 2.05 per cent under the same conditions, and possessed a very disagreeable odor and a nauseating taste. It also showed a considerable increase in color, probably due to decomposed albumin, etc. The change taking place in the sample was demonstrated to be a simple hydrolysis by heat and moisture. In the next experiment, both "B" and "11" (original samples) were dried very carefully by passing through them a current of dry air for seven hours at 100°; 10 cubic centimeters each of the dry oils were then sealed in glass tubes and heated at about 160° for ten hours. For comparison, two more tubes of oil were subjected to the same conditions, one containing 10 cubic centimeters of sample 11 (undried), the other 10 cubic centimeters of the dried oil "B" plus 3 drops of water.

Oil.	Before heating.	After heating.
"B" dry	0.07	0.09
"B" + aq	0.07	14.8
11 dry	0.18	0.19
11 original	0.18	0.46

In neither of the samples marked "B" was a bad odor or any color produced, though the hydrolyzed sample was slightly unpleasant to the taste, owing to the large amount of free acid present. However, both the similarly treated tubes containing oil No. 11, in spite of their low acid value, were decidedly disagreeable to the taste and smell and presented a decomposed appearance.

FACTORS WHICH CAUSE RANCIDITY IN OIL.

The average person, if asked to judge of the quality of these four heated oils by the sense of taste and smell alone, would almost invariably say that both samples of oil No. 11 were "rancid" and that the other two were fairly pure, whereas, judging from the amount of free acid present, one might consider " B + aq." as the only sample containing a marked amount of rancidity. From the above it is evident that rancidity and free acid are not by any means synonymous and that the cause of the former must be sought elsewhere than in the percentage of the latter.

Lewkowitsch applies the term "rancidity" only to those fats which contain an excess of free fatty acids due to the action of air.

Alder Wright [16] gives a résumé of the work done on this subject and concludes

[16] Alder Wright: *Fixed Oils, Fats, Butters, and Waxes*, second edition (1903), 168.

that rancidity is the alteration which oils and fats undergo when not protected from the influence of *air and light*. "Such oils," he says, "acquire a sharp, disagreeable taste and odor, their proportion of free acids gradually increases, and they undergo various other chemical alterations."

Schmid[17] differentiates between "sour fats," "rancid fats," and "sour and rancid fats." "A fat is sour," he says, "when its content of free fatty acids is abnormally high but the free glycerine is unchanged. A fat is rancid when the proportion of free fatty acids is not high but the free glycerine has been oxidized partially or completely to aldehydes and ketones. A fat is rancid and sour when it contains a large amount of free acid together with oxidation products of glycerine." As a test for rancidity he proposes a 1 per cent solution of m. phenylendiamin.

Scala[18] found œnanthylic aldehyde among other substances present in rancid olive oil, and assumes that this body gives the characteristic odor and taste termed "rancidity."

Bianchi[19] proposes, as a test for rancidity, to shake up a little of the oil in question with fuchsin-sulphurous acid, a violet-red color indicating rancidity.

Brown[20] uses this same test in the study of butter fat, and presumes that the rancid odor is due to acroleïn. Various other tests for rancidity have been proposed, all depending on the presence of aldehydes.

The most satisfactory, in my experience, is that with fuchsin-sulphurous acid, shaking up about equal parts of oil and reagent. Nearly all the samples of coconut oil prepared in this laboratory, after standing for several months, responded to this test, but they gave, not a violet red, as has been stated to be the case with other rancid oils, but a more or less blue coloration with only a slight tinge of red. The above review of the literature will demonstrate that the causes of rancidity are by no means clear. Certainly œnanthylic aldehyde has not an intensely disagreeable odor; acroleïn has, but then it gives a red and not a blue color with fuchsin-sulphurous acid.

ACTIVE OXYGEN IN COCONUT OIL WHICH HAS BEEN STANDING.

Another peculiarity of pure coconut oil is that, after it has been standing exposed to light and air for a few months, it almost invariably contains active oxygen. Five cubic centimeters of sample "A," shaken in an Ehrlenmeyer flask with a mixture of 50 cubic centimeters of water, 5 cubic centimeters glacial acetic acid, and 1 gram potassium iodide and allowed to stand for one hour, produced a deep-yellow coloration in the water solution, requiring 0.25 cubic centimeter $\frac{N}{10}$ sodium thiosulfate for decolorization. A blank test, with freshly prepared oil, remained perfectly colorless during the same time. A simple way of performing this test is to saturate a strip of starch iodide paper with the oil in question,

[17] A. Schmid: Zur Prüfung der Fette auf Ranzidität. *Z. Anal. Ch.* (1901), **37**, 301.

[18] Alberto Scala: *Staz. sper. Agrar. ital*, **30**, 613, *Centrbl.* (1898), 439.

[19] *Centrbl.* (1898), II, 948.

[20] Brown: The Chemistry of Butter Fat. *Jour. Amer. Chem. Soc.* (1899), **21**, 975.

place it on a glass plate, and carefully add one drop of 10 per cent acetic acid. When old oil is used, a blue ring appears around the drop of acid in from one to five hours, whereas a freshly prepared sample remains uncolored for twenty-four hours or more, provided the test paper is kept under a bell jar to exclude laboratory fumes. This reaction is at least as delicate as any of the tests for rancidity based on the presence of aldehyde. Whether it is given by other rancid oils can not be stated at present, for I have as yet failed to find in the chemical literature any mention of such oxidizing substances in oil. The following is a table showing the reaction of our samples of coconut oil with fuchsin-sulphurous acid and with starch iodide, together with their age and acidity at the time of testing. For the previous history of these oils, see page

No.	Approximate age, in months, at time of testing.	Approximate percentage F. F. A.	Color with fuchsin-sulphurous acid.	Test for peroxides.
A	15	0.1	Strong blue	+ [a]
B	5	0.1	do	+ [a]
1	5½	1.5	do	+
2	5½	1.5	do	+
3	5½	2.1	do	+
4	5½	5.9	do	+
5	5½	7.6	Blue-red	—
6	5½	0.2	Strong blue	Trace.
7	5½	0.2	do	+
8	5½	0.2	do	+
9	5½	0.2	do	+
10	5½	0.2	do	+
11	5	0.3	do	+
12	5	0.1	do	+
13	5	0.1	do	+
14	5	4.0	No color	Trace.
15	5	0.9	Strong blue	+
16	5	2.0	do	+
17	4	0.1	do	+
18	4	0.2	do	+
19	4	1.2	do	+
20	4	0.7	No color	+
21	4	0.7	do	+
22	3½	23.3	Red	—
23	3½	1.6	Light blue	Trace.
24	3½	1.5	Strong blue	+
25	3½	3.4	Moderate blue	+
26	3½	2.6	Strong blue	+
27	3½	2.4	Light blue	Trace.
28	3½	8.5	Very strong blue	+
29	2	0.1	No color	+
30	2	0.4	Light blue	+
31	3½	3.5	Trace	+
32	3½	7.8	Moderate blue	—
33	7	7.0	No color	—
34	5½	10.2	Strong blue	+
35	½	5.0	No color	+

[a] Very strong.

In examining this table it will be noticed that in almost every case a distinct blue color with Schiff's reagent is accompanied by a positive test for peroxide, and, in the two cases where a red coloration predominates, the peroxide test is negative. The strongest tests for both peroxide and aldehyde were given by samples A and B—two very pure oils—while, contrary to our expectations, the commercial oils as a rule either failed to respond entirely or gave very weak tests, though they were infinitely worse than the pure samples in every other particular. The only change noticeable in samples A and B, on standing, was the development of a peculiar, pungent, "strong" odor and a slight burning "after taste;" otherwise they were practically the same as when freshly prepared. Just what this "strong" odor in pure oils is caused by can not at present be stated, although the subject is now being investigated. It can hardly be caused by either œnanthol or acroleïn, as both these substances, when mixed with oil, give a red and not a blue color with Schiff's reagent. Glycerine aldehyde[21] under certain conditions produces a blue-red coloration with this test, but it has no odor. However, the process by which this change is produced is undoubtedly due to direct oxidation by light and air, since bacterial or mold action may be excluded in the case of a pure oil. That it is largely a surface action is indicated by the facts that (1) samples A and B, which were kept in large bottles, about half full, have deteriorated in five months to a much greater extent than have other samples of pure oil which were kept in small, nearly full bottles for over a year; (2) both the aldehyde and peroxide tests were given by samples of fresh oil which were exposed to the air on strips of filter paper for one or two weeks;[22] (3) the same effect can be produced by treating fresh oil with platinum black for a few hours, or by heating it, exposed in a thin layer, to 100° for ten or twelve hours.

A possible explanation of this production of rancidity in pure oils is that a small percentage of fatty acid is oxidized to an oxyacid, which in turn forms a lactone, and (assuming the formation of hydrogen peroxide) the latter would give rise to a peracid, which, in turn, would oxidize the free glycerine to an aldehyde. The absence of peroxide and, as a rule, of aldehyde in commercial coconut oils, or in those purposely subjected to the action of micro-organisms, may be due to the presence of sugars and other reducing substances commonly present in the impure oils, or to the fact that the glycerine set free by mold action is completely oxidized to carbon dioxide and water. The nonexistence of free glycerine in highly rancid fats has been noted by Sparth[23] and other observers. This question of the products arising from the oxidation of pure coconut oil by air is now being taken up more thoroughly, and the results will be published in a later paper. However, from a commercial point of view, it is of

[21] E. Fischer u. Tafel. *Ber. d. Chem. Gesell.* (1887), **20**, 3384.

[22] Freer & Novy: *Amer. Chem. J.* (1902), **27**, 161.

[23] *Zt. Anal. Ch.* (1896), **35**, 471.

comparatively little importance, when one considers the marked deterioration produced by micro-organisms acting on copra and impure oil. If stored in nearly air-tight containers very little if any oxidation should take place even on long standing or on transportation. The main points to remember are that the copra from which oil is made should be fresh and be prepared under as good conditions of drying as possible and the oil should be thoroughly dried and filtered until absolutely clear. If properly prepared, it should then be capable of shipment without noticeable deterioration. It is obvious that the best results will be obtained by expressing the oil in the country in which copra is dried, and by using the best machinery for preparing the latter.

SUMMARY.

Soil.—In attempting by means of soil analyses to explain why coconut trees growing near the seashore are more prolific than those planted farther inland, it was observed that—

(1) Chemically, there is very little difference in soils from the two localities, those from inland regions being, if anything, a little more fertile.

(2) The salt water from the sea has no influence on trees in its vicinity, as only amounts of chlorine so small as to be negligible were found to be present even at the bases of coconut trees which were actually growing on the beach.

(3) The greater porosity of soils near the sea, coupled with the fact that they are, as a rule, practically saturated with water at a distance of only a few feet beneath the surface of the ground, is the principal reason why they are more suitable for trees like the coconut, which require an enormous quantity of water for their growth.

(4) Although good coconut soils are apparently almost devoid of fertility, yet, taking into account the character of coconut roots and the large area from which each tree draws nourishment, it can be demonstrated that there exists an ample supply of nutriment for their growth.

The nut; age in reference to quality.—(1) The variations among individual nuts is sufficiently great to render exact conclusions from analytical data difficult, but, taking the average of a number of determinations, there appears to be a slight increase in the proportion of meat, copra, and oil in nuts which have been stored up to a maximum time of three months after cutting. Beyond this period there is a decided decrease in these constituents. Nuts taken from the same tree show somewhat less individual variation.

(2) Four series of ten nuts each, of varying degrees of ripeness, showed a marked difference in the amount of copra and of oil to be obtainable from them, the percentage of the oil in a green nut being only about one-half of that which it is when the nut is fully ripe. This ripening process continues to some extent, on storage, after cutting.

(3) Analyses of coconuts from the same locality, but having husks of different color, prove that the color of a nut has very little if any influence on its composition.

(4) The difference between trees near the seashore and those farther inland is solely in the quantity, not in the quality, of nuts which they produce, coconuts from inland regions averaging fully as well as those from the beach. This fact is shown both by analyses and by practical tests on a large scale.

(5) Analyses were made of twenty ripe coconuts from Davao, Mindanao, and they were found to have very much the same proportion of the various constituents and to give the same total yield of oil as the average lot of ripe nuts from San Ramon.

(6) Coconut oil is generally stated to have a great tendency to become rancid, but all the experiments made in this laboratory show that, when once prepared in a pure state, its keeping qualities are equal if not superior to those of most other vegetable fats and oils. This popular fallacy in regard to coconut oil probably arose from the inability or disinclination on the part of most observers to procure pure samples, as the commercial product unquestionably has a high acid value and a bad odor, and deteriorates with fair rapidity, this change being greater as a rule the greater the initial acidity of the oil.

(7) Most of the free acid and the accompanying bad odor and taste is produced in the copra itself before the oil has been expressed. The oil from a sample of copra which had been cut into fine pieces and exposed to moist air for one month increased in acidity from 1.5 to 23.3 per cent.

(8) The hydrolysis and subsequent destruction of fat in copra is brought about by molds (the greater part of which are *Aspergilli*), acting either alone or in symbiosis with certain bacteria, the condition most favorable to this growth being a moderately high, constant temperature and a water content of from about 9 to 17 per cent. No organisms were found growing on a sample containing 4.76 per cent of moisture and no change in acidity took place. Samples containing from 23 to 50 per cent of water were infested by several species of bacteria which subsisted on the nonfatty portion of the copra but produced very little free acid from the oil. No molds were found in these samples.

(9) Ordinarily, commercial copra contains from 9 to 12 per cent of moisture, a very favorable condition for mold growth. The remedy for this rapid deterioration is simply to dry it so that it contains not more than 5 per cent of moisture, and express the oil as soon as possible, avoiding long storage in a warm, moist atmosphere.

Drying.—By comparing the various methods of copra drying, a hot-air apparatus, either rotary or stationary, was found to be the most efficient. It is suggested that a combination of centrifugal with hot-air drying might prove of considerable value, provided a market could be

obtained for the by-product, "coconut cream." Vacuum drying is not of great value in the desiccation of coconuts for oil-making purposes.

(10) Although a pure coconut oil is not a suitable medium for a growth of micro-organisms, one containing a sufficient amount of nutrient matter and moisture may, under certain conditions, develop a growth of mold which rapidly attacks the oil itself. A sample of pure oil to which had been added 1 per cent of "latic" and 1 per cent of water increased in acidity from 0.10 per cent to 8.63 per cent on standing exposed to mold action in an incubator for one week.

The very slight increase in acidity which a pure oil suffers on long standing is probably due to simple hydrolysis by heat and moisture.

(11) Besides the production of free acid by molds and the decomposition of albumen by bacteria in moist copra and in impure oils, one other factor enters into the deterioration of coconut oil. Many samples on long standing develop a slight but noticeably acrid taste and odor, without any marked increase in acidity. Such oils invariably give a blue coloration with Schiff's aldehyde reagent, reduce silver nitrate in Becchi's test for cotton-seed oil, and possess the power of liberating iodine from potassium iodide.[24] This process is shown to be a direct oxidation by the air and to depend largely upon the amount of surface exposed. Other conditions favoring it are freedom from moisture and impurities, as is shown by the fact that impure commercial oils, or those which have been acted upon by mold, do not, as a rule, respond to tests for peroxide and aldehyde, while the most marked development of these bodies is noticed in the purest oils.

(12) The action of light and air on coconut oil is of relatively little importance in comparison with the great changes produced by mold growth, and it can be prevented in a large degree by keeping oil receptacles as nearly full as possible, so as to reduce the amount of surface exposed.

[24] Since writing the above I find that L. Legler [*Pharm. Centr.-H.* (1904), 45, 839] has noticed the same phenomenon in oxidized lard. As a test for active oxygen he proposes to shake the sample with a solution of neutral lead acetate and a few drops of ammonia. A yellow coloration, due to the formation of hydrated lead peroxide, indicates the presence of oxygen. I have applied this test to old coconut oils and find that, with highly oxidized samples, it gives a strong coloration, but it is not as delicate as the simple reaction with potassium iodide. To the presence of active oxygen Legler attributes the reduction of silver nitrate in Becchi's test given by samples of oxidized lard entirely free from cotton-seed oil. I have observed the same fact when applying Becchi's test to pure, but oxidized, samples of coconut oil, but considered it more logical to attribute the reduction to aldehyde-like bodies present in the oil, rather than to the active oxygen.

THE PRINCIPAL INSECTS INJURIOUS TO THE COCONUT PALM (PART I).

By Charles S. Banks.
(*From the Entomological Section, Biological Laboratory, Bureau of Science.*)

Among commercially valuable trees, few are attacked by as small a number of insect pests as the coconut (*Cocos nucifera* L.); but, on the other hand, the destructive action of this limited number is very great.

The trunk of the coconut does not have its important conducting tissues in or immediately under the bark, as is true of the cacao, the coffee, or the mango. For this reason, even though the tree were completely girdled, it would not be destroyed, as would be the case with the plants above mentioned. On the other hand, insects attacking the growing point would soon kill this part, after which the remainder would speedily die, and, in fact, this result is the one which almost always is encountered. Certain insects enter the crown and destroy it; shortly afterwards, the leaves turn yellow, the fruits, if any are present, drop off, and the tree eventually dies. It is therefore clear that any method which prevents attacks of this kind will preserve the life of the tree.

This paper will treat of some of the most important of the insects destructive to the coconut which have been identified, while those the habits and life histories of which are known but the determination of which has not yet been made will be a subject for further study. The observations upon the habits and life histories have been made both in the laboratory and in the field. I wish to take this opportunity of thanking Mr. W. Schultze for his hearty coöperation in this work and for the illustrations which he has furnished.

THE RHINOCEROS BEETLE.

Oryctes Rhinoceros L. (Tagalog, *Uang*).

This insect belongs to the family Dynastidæ, or that of the giant beetles, a group in which, from the standpoint of body weight, the largest of the beetle tribe are found. This beetle is very common throughout the Philippine Archipelago and in other countries of the East (Ceylon, Java, India, etc.) wherever the coconut tree is encountered. All of the

species of the genus *Oryctes* apparently have the same predilection for the coconut and for similar palms. The presence of the rhinoceros beetle is indicated by the large, irregular holes in the trunks of the trees or at the bases of the largest petioles of the leaves of the coconut. These are made by the adult beetles and serve as a means of entrance for other insect pests, such as the Asiatic palm weevil, and also for the admission of moisture, which eventually causes the trunk to rot. The beetles' attacks are confined to the soft tissues near the top of the tree, and holes seen in the trunk below this point date from the time when the growing apex was here located.

No *Oryctes* has ever been found gnawing the hard, old wood of the trunk of the coconut; occasionally adult beetles are found in these old holes, which, however, are used only as a hiding place during the day. In some of them old cocoons which were constructed when the hole was at the crown of the tree are occasionally found, consisting of masses or bundles of fiber. These have been preserved *in situ*, because of the small size of the opening of the burrow. As time goes on, the old holes become enlarged through various agencies, particularly through erosion and decay caused by the entrance of water, so that these bundles of fiber finally become exposed.

Life history and habits.—Like all other members of the family Dynastidæ, *Oryctes* is a vegetable feeder. While it frequently occurs in heaps of decaying vegetation, the larvæ appear to have the greatest liking for the soft, growing point of the coconut, which is the location from which new leaves, the flowers, and, subsequently, the fruit, obtain their nourishment. Therefore, any injury to this part of the tree immediately results in debilitating the whole plant and eventually in its death. The mode of attack most generally encountered is that in which the female has entered between the long stems or petioles of the outside leaves and those immediately subjacent, and then has eaten a hole into the outer side of the inner petioles, which are protected from the light by the external leaf stems. As this beetle shuns the light, its attacks always begin during the night, and by the following morning it will frequently have entered so far into the burrow as to be protected from the light. It then continues its feeding until a gallery of considerable size has been excavated. The habit of burrowing would seem to be not solely for the purpose of laying eggs but also in order to obtain nourishment, as nearly all of the members of this family feed in the adult stage.

The egg.—I know of no record regarding the actual deposition of the egg, nor have I found it in any of the burrows from which the adult has been taken, but, by dissection of the females, the eggs have been obtained in considerable numbers. Just before being laid they are of a dark cream color and present a perfectly smooth texture. The microscope reveals a very delicate reticulation or punctuation of the surface. They are 3.5 millimeters long and 2 millimeters in diameter, being of a perfect

broad, ellipsoidal form. The structure of the ovipositor of the beetle leads one to infer that the egg is simply dropped by the female at any spot in the gallery, where it adheres to the side of the latter until the larvæ are hatched. In none of the females which I dissected were there more than seven or eight eggs of such a size as to indicate that they were about to be laid, so that the insect probably deposits not more than two dozen during its life. (Pl. I, fig. 1.)

The larva.—Length over dorsum when full grown, 112 millimeters; circumference, 18 to 20 millimeters; head, 12 millimeters long and 11 millimeters wide; feet, 9 millimeters in length. It is a very soft, fleshy grub, the skin of which is transversely doubled in numerous folds, so that it is very difficult to differentiate the body segments. The skin is of a dirty, light ocher, the surface being smooth in spots and in other parts covered with patches of very minute tubercles or spines, which give it the appearance of shagreen. The body is covered with numerous, very fine, golden or dull-whitish, curved hairs, which stand out nearly at right angles to the surface. The head is of a much darker color than the body and horny or chitinous in structure; it is hemispherical and so attached to the body that the mouth projects forward. At each side of the head, projecting downward and forward, is a slender, four-jointed antenna. The dark-brown, slender, toothed mandibles, half the length of the entire head, are so placed as to enable the insect to gnaw its way through the plant substance with great facility. The maxillæ are rather conspicuous and are situated next to the mandibles on the under side of the head and on each side of the very inconspicuous lower lip or labium. The maxillary palpi are 4-jointed; those of the labrum 2-jointed. The labrum is transversely elliptical, the sides slightly covering the inner margins of the mandibles. The color of the labrum and that of the clypeus (the trapezoidal portion above the upper lip) is the same as that of the rest of the head. The mouth parts, with the exception of the tips of the mandibles, are covered with golden bristle-like hairs, which serve as tactile sense organs.

This grub has no eyes. The top and front of the head, therefore, present an unbroken surface, which is somewhat shiny and covered with punctures, which are almost confluent; it is nearly destitute of hairs or bristles. (Pl. I, fig. 2.)

Each of the first 3 segments of the body posterior to the head bears a pair of legs. The first leg joint projects downward, while the succeeding ones are inclined outward and forward; the feet are armed with a single blunt claw and densely covered with light-brown bristle-like hairs, more thickly placed at the tips.

The body is curved, so that the length of the ventrum is much less than that of the dorsum. It is folded or transversely corrugated so as to render it difficult to distinguish the 13 segments of which it is composed, this being the easier toward the anal extremity, where the folds are fewer.

Beginning with the first thoracic segment and excepting the second and third, each remaining one to the eleventh bears a pair of dark-brown subcircular spiracles or openings to the tracheal or respiratory system. These spiracles are chitinous in structure and are composed of an outer broken ring of radiating lines and an inner nearly circular portion, which is the true opening. They may be opened or closed at will. Their large size and great prominence is doubtless owing to the fact that the insect lives embedded in a mass of material in which the supply of oxygen is limited.

The last three segments of the grub's body are nearly smooth and only sparsely covered with hair, and in a living specimen the hinder end is semitransparent

and contains a dark mass of material, consisting of the partly digested cellulose fibers of the plant upon which the insect has fed. The anal opening occurs as a transverse slit at the extremity of the body. (Pl. IV, fig. 1.)

Differences of opinion appear to have existed with reference to the destructiveness of the grub of *Oryctes*. Blandford says:

> They are harmless, and live in heaps of rotting vegetable matter or the manure-like inside of decayed palm trees.

Both Mr. Schultze and I have discovered them in large numbers in coconut trees in which the "manure-like" material inside the trunks gave every evidence of having been made by the grubs themselves. One tree, felled in the town of Magdalena, Laguna Province, while still alive and to casual observation fairly healthy, was found to have an inverted cone eaten out at the crown, as shown by Plate III, fig. 1. This contained seven of the grubs of *Oryctes rhinoceros* L. buried in the frass. There was a tunnel, 3 centimeters in diameter, extending down from the apex of the cone for a distance of 90 centimeters through the heart of the tree, and at the bottom of this tunnel was a full-grown grub, which to all appearances had eaten its way to this point. Mr. Schultze observed in Pagsanjan a tree (Pl. II) 5 meters high, the whole of the interior of which had been eaten out from its top to within a half meter of the ground, leaving a shell with a wall from 15 to 20 centimeters in thickness. Within this, at the lower part, was a mixture of water and decayed matter 50 centimeters deep, indicating that the work of *Oryctes* and the Asiatic palm weevil, together with infiltration from the top, had been continuing for a considerable period of time. Within this rotting mass and at intervals up to the crown of the tree were found the fiber cocoons of *O. rhinoceros* L., while from 75 to 100 larvæ of all sizes, from 5 millimeters to the full-grown grub, were removed. The small number of weevils, the large number of *Oryctes* larvæ and pupæ, and the general appearance of the interior of the tree furnished conclusive proofs that the work was that of the insect in question. Leaving these points aside and reasoning from the anatomy of the larva alone, it is evident that it could work in the wood of coconut with great ease, since it is in every way fitted for burrowing there. If it lives only in manure heaps or in decaying matter, it would appear that there would be no necessity for such well-developed and powerful mandibles, nor would the head have to be of such hard material. It is true that these grubs are always encountered in the presence of decayed matter, either in the tree or in manure and other vegetable heaps, but, when found in the tree, it is probable that the decaying material is a result and not the cause of their presence. It is also true that we never cut into a tree until it shows unmistakable signs of insect attack or disease, and therefore do not see the work of the beetle at its incipiency. I have seen larvæ one-quarter grown removed from the burrows made by the adults in small coconut trees, the leaves of which were pulled apart; and I have also observed the

removal of grubs with a piece of bent wire from young coconut trees, although at the time I did not examine the hole sufficiently to note the actual work of the larvæ.

However, it is not to be doubted that these same insects find a suitable place in heaps of decaying vegetable matter, as the grubs have been found in such locations in all stages of growth. In connection with this question, Father Stanton, formerly of the Manila Observatory,[1] makes the following observations:

We have found several live pupæ in a partly decayed stump of *Pithecolobium saman* that had been lying on an old wood pile for months; at another time we discovered one in a neat oval earthen cell within a broken bottle lying in a heap of refuse near a stable; and on one occasion, in a single heap of earth and manure, within a space of 1 cubic yard, we gathered dozens of larvæ in all stages of development from specimens 1 centimeter in length to those of 12 centimeters just about to transform to pupæ together with half a dozen pupæ and as many perfect beetles with their elytra still rather soft, as though the insects had just emerged from the pupal envelope. In this latter case, at least, it appears quite evident that the whole cycle of the metamorphoses of the insect took place right in this small pile of manure or very near to it. For, as many of the larvæ were very young, they could not well have migrated from the interior of a coconut or buri palm, seeing that there was not a single one of these trees in the whole neighborhood. It is evident then that *O. rhinoceros* does sometimes pass its whole larval and pupal existence in the midst of decayed or decaying organic matter, and consequently that the eggs are deposited in such situations. Whether they are also laid in the holes made by the female in the living tree is still to be ascertained, though from the fact that the grubs are sometimes found feeding in the heart of the tree high up near the crown it seems quite probable.

He quotes Señor Vicente Reyes, of Santa Cruz, Laguna, who says:

It is remarked that coconut trees with all the leaves fresh, with blossoms and fruit all in perfect condition, and without any apparent cause, fall to the ground as though a hurricane had cut them down. On being examined it is found that from the roots up to the distance of a meter above the surface they are completely hollowed out, the whole interior having been converted into a mass of sawdust, and ensconced therein are a number of these worms, which have entered from the roots and worked upward, little by little, eating away and living upon the substance of the trunk itself.

In every case where I have encountered *Oryctes* in trees, except in those which were completely hollow, the work evidently proceeded from above downward. Of course, in those which were hollow, the channels of the grubs were found along the inner surface of the shell of the tree, but the evidence thus exhibited was not conclusive as to whether the larvæ had worked from above downward or the reverse.

Father Stanton notes the finding of the larvæ, pupæ, and adults of *Oryctes* in manure and other decaying organic matter, but he also says that he has not ascertained whether the eggs are laid in the holes made by

[1] *Phil. Weather Bur., Bull.*, August (1903), 225.

the adult beetles in the trees, adding that the fact that the grubs are sometimes found feeding in the heart of the tree high up near the crown makes it seem quite probable that the eggs are laid there also. Every evidence in my experience points to the great likelihood that they are laid in these holes.'

When a tree which is so badly infested as to give external signs of debility is cut down, one usually finds larvæ of all stages as well as pupæ. The question of the length of the life period of this insect is a difficult one to determine, but from examinations, such as it has been possible to make during the time the insects of coconut have been under special observation, I am led to believe that it varies from eighteen months to two years, according to the food conditions. These conditions are determined largely by the size of the plant and the proportional number of insects in it.

Pupa.—The pupa of a female measures about 50 millimeters in length and 25 millimeters in width. The distance over the back from the tip of the head to the hinder part of the body, which in the pupa is curved forward, is 65 millimeters. It is of a light ocher yellow, in certain lights presenting a bright satin sheen and in others a velvety appearance. The head, thorax, abdominal segments above and below, and the wings and legs are all plainly visible, the anterior apex of the pupa, at a point corresponding with the top of the head in the adult insect, shows a small sharp knob or tubercle, which represents the horn of the full-grown beetle. A very fine golden pubescence, covering certain areas of the pupal body, causes its velvety appearance. The spiracles are placed similarly to those of the larva, but are almost hidden by the folds or wrinkles of the abdominal segments. On each side of the middle line of the back of the abdomen, transverse slits, very much like spiracles in appearance and undoubtedly secondary breathing orifices, are seen. These occur between each two abdominal segments, beginning with the first and continuing to the seventh, inclusive. Between the seventh and eighth there is indication of their existence in an atrophied state. (Pl. IV, fig. 2.)

Cocoon.—The cocoon is composed of fibers of the coconut, wound transversely and rather compactly woven or matted together. It sometimes measures 100 millimeters in length and 40 millimeters in diameter. When these insects live in rotting material or manure, the cocoon consists simply of an oval excavation, the interior being smoothed by the larva previous to its transformation. Unlike many pupæ of insects which feed in the interior of masses of material, this one has no organs by means of which it may cut or push its way out of the cocoon at the moment of transformation to the adult.

Adult.—The full-grown insect varies in length from 34 to 48 millimeters, according to the sex and the amount of nourishment taken in the larval stage, the average for the males being 44.2 millimeters and that for the females 37 millimeters. They are of a very dark-brown, somewhat lighter beneath, and have a very glossy or shiny appearance. The most striking feature is the horn on the fore part of the top of the head, this being much larger in the male than in the female. The head, thorax, and abdomen are easily distinguished. (Pl. IV, fig. 3.)

Male.—The head, with the exception of the horn, is irregular in form and subglobose; the front is strongly emarginate or sulcate. It is small in comparison with the thorax and so concealed posteriorly by the thorax, into which it fits very snugly, that it appears to be subtriangular from above. The eyes

are black and shiny and so situated at the sides of the head as to be half concealed by the anterior margin of the thorax. The anterior margin of the orbit is extended so as almost to cut the eye into upper and lower sections. The portion of the head at the base of the horn, which extends upward directly from the clypeus, is very densely pilose or setose, as is also the frontal sulcus. The occiput has an emargination at each side of the median line; into these fit the strong tendons of the powerful muscles which move the head upward. The antennæ project from the under, outer margin of the head. They are composed of 11 segments, the apical 3 of which are laminate; the first is swollen at the apex, is as long as the succeeding 7 together, and is very strongly pilose, the bristles being on its anterior, external surface (Pl. I, fig. 3). The ninth and eleventh segments are also pilose at their outer margins and tips; the tenth, lying concealed between them, is smooth and blade-like. The small, 4-jointed maxillary palpi lie just beneath the insertion of the antennæ at each side of the labium, which is subquadrate, with the anterior surface strongly swollen. Its lateral margins are strongly setose. The maxillæ are laminate and hidden between the labium and the peculiarly shaped mandibles. They are strongly setose on their exterior margins. The 3-jointed labial palpi lie beneath or anterior to the maxillary palpi and are attached to the apical part of the lateral margin of the labium.

The most peculiar feature of these insects, which has hitherto been unmentioned in the literature on the habits of the adult, is the special form and function of the mandibles. In the general description of the genus to which this insect belongs, the statement is made that "the mandibles are prominent and sometimes toothed externally." In the rhinoceros beetle the external tooth of the mandible is curved upward and forward and has the form of the cutting edge of a nonconcave gouge. These teeth, one on each side of the head, are by their construction and that of the surrounding parts well adapted for chiseling out the wood of the tree. (Pl. I, fig. 4.)

The shape and position of the external mandibular teeth, the form of the mentum, or chin, which is rounded and curved vertically, and which fits into a groove having a like form, in the anterior margin of the prothorax, together with the strong, well-attached muscles at the back of the head, prove conclusively that the insect, instead of gnawing its way into the tree, chisels into it by an up-and-down motion of the head, and it is my belief, for reasons to be given later, that no part of the wood is taken into the body.

The horn of the male is 10 millimeters in length and 4 millimeters in width at its base, tapering gradually to 1 millimeter at the tip, which in many specimens appears as if worn off and repolished. It is sparsely punctured, these punctures being fewer toward the tip.

Thorax.—The pronotum occupies about one-third of the length of the insect on the dorsum, is roughly subcircular in general outline, narrower anteriorly, having the margins somewhat reflexed. The anterior two-thirds shows a large, shallow depression, the surface of which is transversely or concentrically strio-punctate, and at the posterior margin of which are two rather inconspicuous tubercles, almost coalescing. On each side and in the forward angle of the pronotum there is an irregular depression, posterior to which and extending narrowly around the posterior margin of the main depression, is another parenthesis-shaped one, broader anteriorly and having its surface roughly rugose. A line of submarginal punctures extends around the pronotum. (Pl. I, figs. 5 and 6.)

Elytra.—At the base, the wing covers are as wide as the thorax, the surface at the outer basal angles being quite smooth. On each elytron are four lines, one of which is subsutural, extending from base to apex. The external ones are

very indistinct toward the apex. The part between these lines is coarsely punctured, the punctures being regular on each side of the three external lines and on the exterior of the subsutural ones. The triangular scutellum, between the bases of the elytra, is smooth at its apex. There is a triangular rugose or coarsely irrorated area at its base.

The under surface of the thorax is of a chestnut brown; it is highly polished on the areas against which the legs move and strongly punctured on other exposed parts, the punctures having a sparse pile of light-brown bristles.

Legs.—The femora are uniform in size and smooth, each with 1 row of setose punctures nearer the posterior ventral margin. The tibiæ are nearly similar in shape and size, bearing externally 3 rather prominent teeth. The fore tibiæ, in addition, have an internal and external apical one. The mid and hind tibiæ have each 1 internal and 2 external apical teeth, armed with a row of smaller secondary ones, and all are coarsely punctured. All the tarsi are of about the same shape and size, except that the last joints of the anterior ones are slightly longer than the others, and the first of the mid and hind tarsi are subconical and slightly larger than the succeeding ones. All tarsal joints are setose or spinose at their apices.

Abdomen.—The dorsal abdominal segments are hidden by the elytra; the 6 visible ventral ones are smooth, except for very sparse punctures and a subapical row of setose punctures on each. There is a tuft of brown hairs at the anal slit. The hinder exposed part of the abdomen is rounded, smooth, shiny, and sparsely punctured. The elytra do not cover the last 2 dorsal segments.

The principal differences between the female and the male are that the former is much smaller and its horn may be a mere tubercle, or, at best, not more than one-fourth as long as that of the male. The depression on the pronotum extends back less than halfway; the posterior lateral rugose areas are somewhat broader. (Pl. I, figs. 5, 6). The last ventral abdominal segment of the female differs from that of the male, in that in the former it is rounded and covered with bristly hairs, while in the latter it is markedly emarginate. The ventrum of the abdomen of the female is rather densely covered in transverse rows with bristles, except along the apical margins of the segments. The posterior part of the pygidium is also densely hairy. Both sexes have on all the thoracic joints, as well as at the articulation of the head with the thorax, a fringe of bristles closely applied to the surface upon which the part is attached to prevent the entrance of foreign matter between the sutures.

Method of operation of the adult.—The method of attack of the adult insect was formerly believed to consist in its gnawing into the plant for the purpose of feeding upon the soft tissues inside, its eggs not being laid in the tree. This view is partly incorrect. Observation has shown that the males make burrows, as well as the females, and it is probable that they always accompany the latter at the time of egg-laying, retreating from the burrow they have made to allow the female access. Dissection demonstrates that the stomach of the insect contains no masticated fiber; on the contrary, it is filled with a dark, amber-colored liquid; nor are there any fiber cells found in the excreta. The proventriculus or gizzard of the insect is not provided with walls for grinding and the mandibles are constructed somewhat like the parts of a cane mill through which the sugar cane passes in expressing the juice, except that their surface is corrugated, the elevations of the one fitting into the depressions of the other.

The œsophagus is not more than 1 millimeter in diameter. The insect begins the process of separating the fibers of the tree by means of its chisel-like teeth. The rapidity with which a beetle can work is shown by the fact that within half an hour it will have entered a fourth of its own length into the plant tissue, and when once it is enabled to brace its strong-spined legs against the walls of the burrow its progress is accelerated. The heart of the tree is its objective point.

On Plate V are shown successive layers of leaf petioles at the heart with the burrow of an adult which finally reached the center. Figs. 1, 2, 3, and 4, respectively, show the pieces from outside, inward.

An examination of the fibers as soon as they are cut by the beetle demonstrates them to be almost dry, which renders it more probable that the purpose for which they are taken into the mandibles is solely to extract their juice, after which they are expelled from the mouth. Plate V, fig. 4, shows the heart of the coconut tree with a beetle at work. The bits of tissue which have been chiseled off can plainly be seen. In less than ten minutes the insect had burrowed into the soft substance for a distance of 10 millimeters. Plate III, fig. 2, shows the initial work of a beetle in a leaf petiole.

The beetles fly only at night; in the daytime they are readily found in their burrows. Their wings are quite large and the wing muscles in the thorax are strong and adapted for the flight of such heavy, unwieldy insects. In the interspaces between the intestines and the reproductive organs the abdomen is filled with air sacs and tracheæ.

Extent and character of damage done.—It is rare to find a single coconut tree anywhere in the Philippines which does not show one or more evidences of attack by this beetle. It is the pest most frequently reported by farmers and coconut growers, and in hundreds of trees which I have personally examined large holes in the trunk, distorted leaf stems, or ragged leaves demonstrate the character of its work. The insect larva or the adult, in its work inside the tree, frequently cuts off the tip of the embryo leaf or the tips of the leaflets on one or both sides of the midrib, so that when the leaf finally grows it appears as if it had been trimmed with a pair of shears or as if a triangle had been cut from one or both sides. The fibers severed by the insect protrude from its burrow, giving the latter a ragged appearance. During the daytime the beetles are frequently encountered in very old holes, into which they evidently have gone for the purpose of hiding. They have never been seen further to excavate these old cavities. The openings which are made serve to allow rain water to enter the tree, where it causes a most rapid decay of the interior, and they also serve as an entrance for other insects quite as destructive as the coconut beetle.

Distribution.—*Oryctes rhinoceros* L. is probably tropicopolitan, being found in Honduras, India, Ceylon, Java, the Philippines, Celebes, Borneo

and Sumatra, and recorded as coming from Africa. It undoubtedly thrives well wherever the coconut is grown.

Dr. Köningsberger, of Buitenzorg, Java, says: "The well-known coconut beetle *O. rhinoceros* L. is one of the most terrible enemies of coconut culture." And if this be the case in Java, where cultural methods have been in vogue for so many years, it is probably much truer in the Philippines.

Preventive and remedial measures.—The question of controlling the ravages of this insect is a difficult one. It would appear that trees growing to such a height as the coconut and having so few parts would not be seriously affected by a rank growth of weeds or underbrush or by a lack of cleanliness in their surroundings, but this is certainly not the case. It has already been stated that the larvæ of the coconut beetle grow in manure and rotting vegetable heaps and also thrive in rotten or rotting coconut trees, their abundance appearing to be in direct ratio to the degree of decay which the tree has attained. Mr. Schultze has taken as many as a hundred larvæ of all sizes from the decayed shell of a coconut trunk. I have invariably found them in great abundance in such situations. It is obvious that these sources of infection for healthy trees must be removed. The first thing to do in coming into possession of a coconut grove or in planting a new one is thoroughly to clean the ground. All manure heaps, rubbish, rotting or fallen trees should be removed and destroyed at once. The manure should be scattered where it will serve the best purpose as a fertilizer, and in such a manner as to make it impossible for the grubs to find lodgment in it. Rubbish heaps and decayed trunks, if fallen, should be burned; if standing, should be cut down and burned. The residue can easily be returned to the soil as fertilizer.

Growers should not remove the dead leaves from their trees to such an extent as to leave the young and still tender petioles or leaf stems entirely exposed, thus inviting attack by the adult beetles. These leaf stems have a thorough natural protection by being wrapped in a woven fiber, the old stems remaining upon the tree until the new ones are fully grown. When the living leaf stems are cut off a foot or two from their union with the trunk, the sap running out offers an attraction to beetles which might otherwise not attack the tree. Blanford discusses this danger as follows:[2]

"The trees should be left, as far as possible, in the natural state, and unnecessary trimming either of fronds or of the fiber avoided. It may be necessary to tie up the older fronds, and, if they must be removed, the stalk should be cut through sufficiently far from the stem to leave the sheathing base intact. It may be advisable to tar the cut stump if it is found to attract beetles. The value of leaving the trees alone is shown by a passage in Ferguson's *All about the Coco-nut Palm,* which is also quoted by Ridley: 'Scores of instances might be recorded

[2] *Kew Bulletin* (1893), 73, 46.

where, till the trees were come into bearing, a red beetle was never seen, but no sooner was the land cleared and the trees trimmed than it made its appearance and became very destructive. On one property the trimming system had been carried on for years, till, indeed, more than one-third of the original plants perished, before the estate was 10 years old, and they were going at the rate of three trees weekly. The work of trimming was stopped for the reasons offered above; the loss of the trees continued for some time afterwards, but at the end of six months it had entirely ceased. On another property beetle men had been employed for ten years, and trees were being constantly lost; from the day that the beetlers were discontinued two trees perished within a month, and not another was lost in the subsequent seven years.' And W. B. L. writes in the *Tropical Agriculturist* to the same effect: 'The red beetle (*Rhynchophorus ferrugineus*) can not penetrate the leaf imbrication, and, when the older ones decay in the course of nature, the stem has become too hard for its operations. A tree here and there may be lost from an accidental wound or from some defect in the fitting of the leaf sheaths, but it is only where the good taste of the planter has impelled him to trim the leaves that any serious damage has been done to a field. All the leaves should be left on the tree till nature disposes of them at her own time and in her own way. Nothing that can be done to a coconut tree above ground can be anything but injurious.'

"All wounds, whether made by accident or by insects, on the soft part of the stem, leaf sheaths, or spike should be at once dressed with a dab of tar mixed with fine sand. Holes should be probed with a "beetle spear" or hooked wire to extract insects which may have caused them and then plugged with a tuft of fiber or dry grass dipped in tar."

Obviously, no tree should be condemned until a careful and thorough inspection makes it certain that it is beyond hope of recovery and that it can bear no more fruit. It has been suggested by certain writers that a good plan is to cut such felled trees open, leaving them on the ground to attract beetles which would otherwise fly to the healthy trees; but I am of the opinion that the less material of this kind there is in the orchard the less is the liability of attack incurred by the bearing individuals. If there are no wounds or vulnerable spots in the trees themselves, and if nothing remains on the premises to attract this beetle and others, the less will be the danger. The dead leaves should be allowed to fall in the natural course of growth and care should be taken not to mutilate the trees. However, in most instances the beetles already have invaded the plantations and the serious problem is how to rid these places of them and to prevent their reëntrance. Of course, frequent inspections are necessary, so as to detect invasions at the earliest possible moment, because, as the coconut beetles hide in their burrows during the day, it is comparatively easy to destroy them if they are noticed in time. When they are discovered, a long, hooked steel wire can be thrust into the burrow, given a half turn to engage the insect upon its point, and then drawn out. This operation requires some practice, as the beetle is well armored with a smooth coat and has few projections upon the body. Dr. Köningsberger suggests crushing the insect and leaving it in the burrow as an obstacle to the entrance of others; but this is not to be recommended in

the Philippines, because the dead material would be sure to attract ants, which in turn would draw other insects, such as white ants (*anay*) and serious complications might arise. When the insect has been killed and removed, it is essential to plug the hole with some substance which will prevent further attacks at that point. For this purpose various substances have been recommended, for example: tar and fine sand, plaster and sand, clay and tar, or, in place of clay, plaster or cement. This mixture should be forced into the holes as far as possible, because it then will act as a deterrent to the decay caused by the entrance of moisture subsequent to attacks of the beetle, while effectually closing an avenue of entrance for others. Another remedy is to use a paste of Paris green and flour, mixed with 10 or 12 gallons of water, and sprayed into the crown of the tree. This method would offer difficulties when tall trees are to be dealt with, owing to the impracticability of getting the spray to the right places. The Filipinos use various remedies, such as sand and coarse salt, which they place in the crown of the tree. They state that the sand gets between the articulations of the head and thorax of the beetle, where the constant friction sets up an irritation which eventually punctures the soft tissues, after which the insect dies. This may be true. There is a constant movement of the head and of the thorax, while the beetle is working its way into the tree, and although the articulations are protected, as explained above, by a fringe of closely fitting bristles, it is possible that fine sand might enter as suggested and thus seriously handicap the beetle in its boring operations, if not eventually killing it.

I have been assured by a gentleman who is one of the most successful farmers in the Islands that natives on his plantation pour urine into the crown of the affected coconut trees and that this method effectually rids them of the pest. This is certainly not impossible.

ASIATIC PALM WEEVIL.

Rhynchophorus ferrugineus Fabr.

"It has been observed that coconut palms, the green leaves, blossoms, and fruits of which appear in perfect condition, fall to the ground, without having any signs of decay, as though struck by a hurricane. In such instances it has been noted that (the trees) from the roots up to a meter in height, are completely undermined, the interior pulverized like sawdust and filled with nests of these worms, which have gained entrance through the roots and gnawed their way upward, deriving maintenance from the trunk." [a]

The gravity of the attacks of the Asiatic palm weevil is well summed up in the foregoing extract, for, while the condition referred to is not generally reported from all parts of the Islands, there is every reason to

[a] Extract of translation of a communication from Señor Vicente P. Reyes, of Santa Cruz, Laguna, with reference to *R. ferrugineus* Fabr.

believe that in general the depredations of the beetle are no less serious in their ultimate effects than in the cases reported from the Provinces of Laguna and Tayabas, in which regions Señor Reyes has seen the damage to which he has made reference. I visited Magdalena, Province of Laguna, which lies in a fairly productive coconut region, and have found conditions closely resembling those set forth above, except that only a very few trees has actually fallen. In most instances where this had occurred the stumps had been cut off rather close to the ground during the previous year, and hence we found little material at hand upon which to work. However, we were convinced that the menace to coconut growing from this insect is fully as serious as, if not more so than, that occasioned by the attacks of *Oryctes rhinoceros* L.

This weevil enters the tree through the smallest wounds, leaving no external trace of its work, so that all its ravages are committed where not suspected; hence it is an extremely difficult enemy to combat.

The Asiatic palm weevil belongs to a group the members of which are, almost without exception, destructive to vegetable substances, either living or in the form of stored products, such as grains, beans, pease, and nuts. The beetle under discussion is one of the largest of its kind. The rice weevil is not more than 5 to 6 millimeters long; the corn weevil, *Calandra oryza* Linn., is slightly larger; the boll weevil, *Anthonomus grandis* Boh., which is at present proving so serious a menace to cotton growing in the United States, measures about 5 millimeters in length; the plum curculio, *Conotrachelus nenuphar* Hbst., a weevil, is 6 millimeters long; while there is another species attacking the coconut which measures 13 millimeters. The Asiatic palm weevil measures 35 millimeters in length. The form is strikingly characteristic in all individuals of this group. The most prominent features are an oblong, oval-shaped body and a long, slender, curved snout or bill, to which are attached the antennæ, either near the base or the tip. The colors vary from black to light brown or red, but are usually obscure.

The larvæ are legless, with a head of chitinous or horny structure, usually darker than the body and having two strong mandibles well adapted to gnawing the hardest vegetable substances. The Asiatic palm weevil has never been seen to make a primary attack upon the hard wood of the coconut; wherever it has been observed, it has utilized the holes made by *Oryctes*, wounds carelessly made around the base of the tree, or the steps cut into the sides of it by the tuba gatherers. Wherever the hard bark is broken and the softer parts beneath exposed, excellent places for the laying of the eggs are found and the beetle often makes a hole 10 millimeters deep before depositing them. The character of the hole and the tracks of the larvæ after hatching are shown diagrammatically in Plate VI, fig. 4. In laying their eggs in the burrows made by *Oryctes*, the palm weevils undoubtedly make no appreciable hole, simply

forcing the egg a short distance into the soft material in which the burrow lies.

Egg.—The egg is slender, 2.4 millimeters long and 0.6 millimeter wide at the middle, slightly more pointed at one and than at the other, and of a very light ocher. The shell appears perfectly smooth and shiny, but when examined under the microscope the surface is seen to be finely reticulated. (Pl. VI, figs. 1 and 1 *b*.)

Larva.—The larva does not vary essentially in general characteristics from the time of hatching until it is fully developed. Full grown, it measures from 35 to 55 millimeters in length and from 18 to 22 millimeters in diameter. The greatest diameter is slightly behind the middle. The hinder part of the body forms a concavo-convex extension, a blunt spoon or scoop-shaped organ.

The *head* is from 10 to 12 millimeters long and 7.5 to 8 millimeters wide. Seen from above, it is of a regular oval outline. It is of a dark-chestnut brown, with a slight reddish tinge, and with a lighter median and 2 submedian narrow stripes marking the sutures. (Pl. VI, fig. 2.)

The space around the mouth parts and the latter themselves are of a dark brown, with the exception of the upper lip and clypeus, which are lighter. The triangular portion of the head, immediately above the mouth, is transversely rugose, with a longitudinal furrow on each side of its middle. The remainder, including the cheeks and occiput, is engraved with very shallow reticulations, giving the appearance of a piece of alligator skin in miniature. The smooth, dark-brown, subtriangular, rather blunt mandibles are exposed on each side of the mouth; the upper lip, or labrum, lying between them, and reaching nearly to their tips, is provided with numerous bristle-like hairs. The larva has no antennæ, but the maxillary and labial palpi are well developed and doubtless serve as feelers. It has no eyes. The underlip, or labium, is subtriangular and rather small, but quite fleshy; the palpi project conspicuously from each side of its tip. (Pl. VI, fig. 3.) It is supposed that the surface of all these organs is highly sensitive and that the insect can tell desirable food by touch. The head shows erect hairs placed at regular intervals, 5 on each side of the top and 3 back of each mandible. Portions of the front of the head, and the mandibles, appear as if having been rubbed off by friction with the substance in which the larva lives, so that these parts have a dull, almost black or *matte* appearance.

The *body* is composed of a series of 13 rings very much folded and wrinkled, the surface being of a smooth, velvety texture, except in certain spots, which are decidedly shiny. On the back of the first segment appear 2 transverse, oblong patches of a darker color than the remainder of the body, with a surface similar to that of the head and serving as a protection to the animal in its movements in the small galleries in which it works. There are similar lighter areas at the sides of the first 3, or thoracic, segments, which are somewhat swollen and serve in lieu of legs. Scattered over the entire surface are tiny, circular or irregular shiny areas, from each of which arises a small curved bristle. On next to the last segment of the body, dorsally situated, are also irregular shiny patches, each with 6 bristles. The last segment has the upper surface concave, and the lower convex; the posterior margin, which is slightly darker than the rest and smooth and shiny, is flattened out and has four prominences, from each of which project two rather long bristles. The spots from which they project are lighter in color. The wrinkles of the body are nearly symmetrical. (Pl. VII, fig. 1.) The body curves downward, so that the back is very convex, while the underside is somewhat concave, except just back of the middle, where it is convex, then suddenly tapering to the tip.

This grub works its way forward through its burrows by a combination of twisting and undulating motions. In this it is aided by the tubercular enlargements on the thoracic segments. The hind end appears to offer no help in this respect. It can enter from any opening through which the head will pass. The bristles on the head serve as guides for the insect in passing through holes. When placed upon a level surface, the grub moves slightly sidewise, almost always upon its side, and can thus make fairly rapid progress.

The breathing apparatus in *R. ferrugineus* Fabr. consists of only two pairs of spiracles which are well developed, the others being almost rudimentary. Each of the first pair is situated laterally on the first thoracic segment, and twice its own length below the shiny, transverse, shield-like areas, and the second pair just above the spoon-shaped excavation of the thirteenth segment of the body. The latter two are one and one-half times as large as the first two, and their openings are nearly vertical, diverging slightly below. The other segments of the body show the spiracles only when examined under a strong lens; these are nonfunctional, or at most only very slightly so.

The galleries of this grub run obliquely through the large swollen part of the tree near the roots. (Pl. VIII, fig. 3.) The specimen here depicted was full of grubs of all sizes and contained one or two pupæ as well. Adult beetles were also found in considerable numbers. The grubs have been encountered in the crown of the tree in numbers varying from 15 to 20, where they work side by side with those of the rhinoceros beetle, and it is very difficult to distinguish the galleries of the one from those of the other. Plate III, fig. 1, shows a longitudinal section of the crown of a tree which has been eaten out in the form of an inverted cone by the larvæ of the rhinoceros beetle and the palm weevil in company. Plate VIII, fig. 3, shows the work of the weevil in the lower part of the tree very near the roots, some of which are seen at the lower right-hand corner. It will be noted that the galleries run obliquely, which shows that the grubs work inward and upward from the outside of the tree. In this case the eggs were evidently laid in wounds in the root region, on the left, and the grubs worked their way toward the center where the full-sized galleries are seen.

Pupa.—When the larva of the palm weevil has attained its full size, it ceases feeding and evacuates the alimentary canal, thus causing a shrinkage of one-third in its size immediately before making its cocoon. This is elliptical in outline, from 8 to 12 centimeters long and 5 to 6 millimeters in diameter, and composed of the long tough fibers of the coconut trunk wound as shown by Plate VII, fig. 3. It is closely woven and thick, so that the pupa is well protected against dampness. The grub sheds its skin and takes the form shown by Plate VII, fig. 2. The pupa measures 35 to 40 millimeters in length and about 15 millimeters across its widest portion. The snout is doubled down on the breast; the antennæ, wings, and other organs of the beetle are plainly visible. The color is a uniform pale-ocher, the tips of the knees being darker. Rugose areas are situated on each side of the head, back of the eyes, on the upper part of the snout, on the outer fore and hind regions of the pronotum, on the ridges of the elytra, and on the dorsum of the abdomen. These areas are rather thickly

set with short, sharp spines, which aid the beetle in escaping from the pupal skin by holding the latter firmly in the cocoon. The spiracles, which on most of the abdominal segments in the larva were nearly obsolete, are very prominent in the pupa, a pair on each of the first 6 segments and a fainter one on the seventh being visible.

Up to the present it has been impossible to ascertain the length of time of either the larval or the pupal stage. The beetles begin their work in the trees at practically the same time as *Oryctes* and the adults are found together with those of the latter, so that the life periods in the larva and pupa of both are probably about the same, or from 18 to 24 months.

Adult.—*Rhynchophorus ferrugineus* Fabr. is an extremely variable insect, in its markings as well as in its size. Specimens have been obtained of from 25 to 35 millimeters in length, while the color varies from a true ferruginous, with certain black markings more or less regularly placed, to almost entirely black, with only traces of ferruginous. (Pl. VIII, fig. 1.)

***Rhynchophorus* sp.**

This species is very closely related to *R. ferrugineus* Fabr., if not identical with it, merely varying in general color and in having a broad, ferruginous, longitudinal band from the front to the hinder margin of the thorax. (Pl. VIII, fig. 2.)

The habits and the immature stages of this insect are similar to those of *R. ferrugineus* Fabr. These beetles are found indiscriminately in company on the same tree, and no differences are noted until the adults are compared.

Preventives and remedies.—The prevention of the first attack of the pest is essential. The adult male or female can not bore into the solid tissue as can that of the rhinoceros beetle, because the snout is small and the mandibles are relatively weak. For this reason the female seeks wounds or holes of any kind in the trunk of the tree to deposit her eggs. These wounds may have been caused by other insects, or they may be accidental. One of the chief injuries to the trunk of the tree is that caused by the gatherers of tuba, who make notches in it whereby they may be enabled to climb to the top. As these notches are of considerable size and depth, they offer excellent facilities for the beetles to enter and hide or lay their eggs. All such mutilation of coconut trees should certainly be prevented, even if it be necessary to construct bamboo ladders, securely fastened to the trees, as is done in some localities. There are frequently encountered in coconut plantations trees the bases of which seem to have been chopped with no apparent purpose in view. Of course, these offer an excellent opportunity for the beetles to begin their work. A good, healthy, vigorous, uninjured coconut tree is practically invulnerable to the attacks of the palm weevil.

If, in spite of all precautions, the weevils gain entrance to the tree, the work of combating them is exceedingly difficult. Frequently, when

they are in the softer, upper parts, it is possible to dig them out with a wire hook similar to the one mentioned as effective against the rhinoceros beetle larvæ. In every case where these or other larvæ are dug out of a burrow, this should at once be filled with a substance distasteful to the adult beetles. Great care is necessary in the work of extracting the larvæ, lest it should be carried to such an extent as to debilitate or kill the tree. If the weevil larvæ are located at or near the base of the tree, where it is almost impossible to dig them out, the only practical method is to stop all avenues of escape and then to remove the tree after it ceases to bear fruit. It has been suggested that infested trees be cut down, split lengthwise, and then left to attract beetles from the others. I am opposed to such a procedure, because it would surely attract fully as many insects from a distance as it would from the immediate neighborhood. Such a method would be advisable only if the other trees were in great danger from beetles already present in them. Plate II shows a coconut tree very badly infested by both the rhinoceros beetle and the Asiatic palm weevil. It will be seen that the entire interior has been eaten out and converted into a mass of débris, in which both the cocoons and the larvæ of these insects were found in great abundance. The tree had ceased to bear, the growing point was gone, and there remained only a circle of older leaves, kept alive by the small flow of sap in the outer shell of the trunk. It is obvious that such a tree is a source of infection for a large area.

THE SHOT-HOLE COCONUT WEEVIL.

This destructive weevil has been found in Laguna Province in considerable numbers. I once felled a dead coconut tree, the trunk of which was completely pitted from top to bottom by the insects' exit holes, and Mr. Schultze found the larvæ and pupæ as well as the adults in a living tree. (Pl. X, figs. 1 to 5.)

Egg.—The egg has not been found. It is probably laid directly in the hard wood in small cavities made by the female, as the grub can work in any part of the trunk of a tree.

Larva.—The larva is a very pale-yellow, almost white grub, measuring 16.5 millimeters in length and 6 millimeters in diameter, resembling the larva of the palm weevil, except that the hinder end of the body is evenly rounded. The head is shiny and but slightly darker than the rest of the body, the region around the mouth and the mouth parts appearing dark-brown. A very thin, brown median line runs from the upper lip halfway to the back of the head. The spiracles are very small and almost black. The surface of the body is smooth and very much folded. A few bristle-like hairs are seen on the head. (See Pl. X, fig. 1, illustrating a full-grown larva.)

Pupa.—The pupa is 13.5 millimeters long and 6 millimeters in diameter and of the same color as the larva. The surface is smooth and shiny. On the head, thorax, and dorsum of the abdomen there may be seen a series of stout, brown bristles arising from brownish tubercles. The tip of the abdomen has a small, white tubercle on each side, from the point of which arises a small, black bristle. There is little difference between the size of the tubercle and that of the bristle.

The pupa rests in a cavity in the live wood, there being no attempt at forming a cocoon. (See Pl. X, fig. 3, giving a lateral view of the pupa.)

Adult (Pl. X, fig. 4.).—The adult both in form and in size appears very much like the willow weevil, *Cryptorrhynchus lapathi* Linn., of the United States, except that there are no tubercles on the thorax and wing covers. It measures 11 millimeters in length (exclusive of the bill, which normally is doubled under the body) and is 5.5 millimeters in its greatest breadth. (Pl. X, fig. 4.) It is of a dark-reddish brown, somewhat mottled with gray on the forward part of the thorax, which is very closely punctured. The head is globular and fits almost entirely into a cavity in the front of the thorax, so that, when seen from above, it has the form of a thin crescent. The eyes are black and somewhat oval, nearly meeting on the front of the head, the space between being one-fourth the width of the rostrum or bill. When the insect is at rest the antennæ, which are inserted on each side of the rostrum near its base, are completely hidden, being drawn within the cavity in which the head fits. They are geniculate, the apical part or flagellum being somewhat more than half the length of the bill, very slender at its base, and increasing in size toward the club-shaped apex, which has three segments very closely united. There are 12 segments in the antennæ, of which 11 are in the flagellum. (Pl. X, fig. 2.) The surface of the 3 apical ones is very pilose and of a sensitive nature. The rostrum is smooth, closely punctured, and slightly broadened at its apex. The mandibles are plainly visible, slightly darker than the rostrum, and uniting at their apices to form a triangle. The part immediately above the mandibles is covered with strong, light-brown bristles, pointing toward the tip of the rostrum. The thorax bears a longitudinal depression which is light-gray in color, owing to the scales on the surface, and which extends nearly to the posterior margin, where the depression becomes a ridge or carina one-sixth the length of the thorax.

The elytra reach nearly to the tip of the abdomen, are very rough, and are traversed longitudinally by nine rows of punctures forming very deep grooves; six of these extend to the apex of the wing covers, the others being interrupted or running into each other. The external (ninth) row terminates before the middle of the elytron. The posterior portion of the propluræ shows a decided depression, into which the front legs fit on each side.

The *legs* are moderately long and very stout, the fore pair being nearly a half longer than the other two. The rostrum reaches beyond the insertion of the first pair; there is a transverse carina of the mesosternum against which it rests. Two spines are situated on the under sides of the femora near their apices, the smaller nearer the apex, and those on the forelegs larger than those of the middle and hind pairs. The tibiæ are all of the same shape, each bearing a curved spine or tooth and 3 bristles at its apex; the latter are external.

The *tarsi* are 4-jointed, the fourth being very small and hidden between the pulvilli or pads. The tarsal claw is bifurcated, very long and slender. The tarsi are covered with long, blunt, silvery-white scale-like hairs.

The exposed part of the pygidium, or hinder segment of the abdomen, is bluntly, almost emarginately rounded; the apical half is covered with golden-brown bristles lying flat. The last ventral abdominal segment is hairy apically. The beetles appear to present no external sexual characters.

Remedies and preventives.—Doubtless these insects would be susceptible to the same general treatment as that given to the Asiatic palm weevil, although too little is known of their habits to be certain. They have been found in all stages, generally in diseased trees or in those debilitated by the attacks of other insects, and hence should not form a serious menace.

THE BONNÑGA WEEVIL.

Cyrtotrachelus sp.

This weevil lives principally in the trunks of the betel palm, *Areca catechu* Linn., where it does great damage, but inasmuch as it has also been found in considerable numbers in coconut trees, it is here described as far as its habits and appearance are concerned. In addition to the larvæ of the rhinoceros beetle and the cocoons and grubs of the palm weevil, one frequently encounters in decaying betel palms or coconut trees of 6 to 8 years, other smaller cocoons not more than 35 millimeters long and 15 millimeters in diameter. (Pl. VII, fig. 4.) These are composed of a more finely comminuted fiber than those of the palm weevil, and upon opening appear to contain a dwarfed example of the pupa of the Asiatic weevil. However, this pupa differs in many respects from that of *Rhynchophorus ferrugineus* Fabr., and the frequent finding of beautifully marked weevils of very small size convinces one that these cocoons and pupæ belong to the former.

Egg.—No eggs have been encountered, and attempts at confining the adults for the deposition of eggs under conditions as nearly natural as possible have failed.

Larva.—The full-grown larva is nearly of the same size as the preceding one. However, in form it is more like that of the Asiatic palm weevil and is probably somewhat closely related to it. The color is a light-ocher yellow. The head is very much darker, and the mouth parts are dark-brown.

The length is 20 millimeters and the diameter 6 millimeters near the rear third of the body, the form being strikingly like that of the Asiatic weevil in this particular. The head projects forward and is smooth and shiny, with but few hairs scattered over it.

The spiracles on the first thoracic segment are larger than any of those on the other ones of the body, with the exception of the last abdominal segment, on which they are well developed and placed on the posterior aspect. The apex of the last segment is somewhat flattened and its hinder margin is prolonged into 4 rather obscure tubercles, from each of which arise 2 bristle-like hairs pointing posteriorly and slightly downward. Certain areas on the skin of the entire body, except the head, are rough or very minutely shagreened, single isolated hairs arising from some of them.

The mouth is minute, but the upper and lower lips and the mandibles are well developed, the latter being black. The palpi are prominent. As with the larvæ of *Rhynchophorus ferrugineus* Fabr., there are no evidences of external eyes or ocelli. (Pl. XI, fig. 1.)

Pupa.—The pupa is illustrated by fig. 3 on Plate XI. Its length is 13 millimeters and its greatest diameter 6 millimeters. It is of a whitish-ocher color, certain of the tubercles being a darker ocherous. On the front of the head, just above the point where the eyes would appear in the adult, there are 2 prominent corrugated tubercles, each with a single bristle; anterior to these are 2 smaller ones with bristles; and on the snout or rostrum above the antennæ are 2 more. On the front margin of the thorax are 2 other tubercles, smaller than the largest on the head; on the anterior half of the thorax toward the sides is another pair; and near the posterior margin are 2 others slightly larger; all of these are provided

with a single bristle. The first 6 dorsal abdominal segments are very sharply defined and bear on each side of the middle a transverse group of 3 tubercles with bristles; outside of these is a single one.

The spiracles are plainly seen at the latero-dorsal angle of each segment. The pygidial segment is curved downward and at its middle there is a transverse line of 8 tubercles with bristles, slightly separated on the median line. The apical part of this segment has a median, transversely corrugated carina. On the extremity of the last ventral segment, on each side, there is a concentrically corrugated tubercle, from which 2 yellowish ochraceous bristles arise.

Each *femur*, on the outer part of its apex, has a single tuberculated spine, darker than the surface. The pupæ are very active when taken from their cocoons, wriggling continuously if held in the hand.

A peculiar large, button-shaped spiracle may be seen on each side just behind and a little below the prothoracic shield.

Adult.—This insect is graceful in form and very delicate in color. A black and white drawing, such as fig. 4 on Plate XI, of course can not show its coloring, which is one of its most striking features. The profile is shown by Plate XI, fig. 6.

The length is 17 millimeters from the tip of the snout to the tip of the abdomen, and the diameter about 4.75 millimeters. Seen from above, it measures 13 millimeters in length. Its color is a combination of ocher, reddish-ocher, and dark-brown, or black, in which reddish-ocher predominates.

The *head*, exclusive of the snout, it globular, and smooth above and below, with a few scattering shallow punctures at the side. Its color is reddish-ocher. The eyes are jet-black and broadly crescent-shaped, nearly uniting at the upper and under sides of the head; their exterior outline, when viewed directly from in front, is almost a perfect circle. The rostrum is cylindrical and strongly curved downward, the basal third being twice the diameter of the rest and covered with circular light-gray spots, from each of which arises a tiny, dark-brown tubercular spine. The apical two-thirds is smooth and finely punctured longitudinally. The tip is slightly swollen laterally and of a darker color, as are the mouth parts. The mandibles are black and glossy, and tridentate, the teeth of one fitting into the interstices of the other. The narrow transverse labrum, with the anterior margin rounded, is scarcely visible. The antennæ, apparently composed of 8 joints, of which the first is equal in length to the others combined, are placed in short deep furrows on each side of the snout a little less than one-fourth the distance from its base. The upper edge of this furrow projects at its middle and somewhat overlaps the articulation of the first antennal joint. The last joint is greatly swollen, being twice the diameter of the preceding one, and is securiform. The length of the antennæ is equal to that of the rostrum. The third joint is chalice-shaped and one-half longer than the second one, which is inserted on the inner apical portion of the first. The prothorax is subconical, three-fourths as wide as the elytra, perfectly truncate at its anterior margin, and slightly rounded posteriorly. A narrow collar extends around its entire anterior margin, the sides of which are subparallel. The sides of the thorax are rounded, and their surface is smooth, dull, and sparsely punctured, the punctures toward the sides bearing small tubercles or spines. These punctures are also found on the underpart of the pro- and mesothorax, the metathorax, the ventral surface of the abdomen, the pygidium, the femora and the tibiæ of all the legs.

Thoracic markings occur as follows: A lancet-shaped, light-ochraceous or buff median mark, extending from a point behind the anterior margin one-fifth the length of the thorax, to the posterior margin; on each side of this a wide black line, broadening perceptibly at the posterior margin, meeting in front of the

median mark and extending to the anterior margin; outside the black lines, on each side, a reddish-ochraceous one, twice as broad as the median and not extending to the anterior margin; external to these on each side, a broad pale-ochraceous or buff line, mixed with reddish-ocher at its outer edge and running imperceptibly into the extreme outer longitudinal black lines on the sides of the thorax. The black lines are irrorated with buff and have buff punctures anteriorly.

The scutellum, between the bases of the elytra, is lancet-shaped, black, and shiny.

The *elytra* have a ground color of reddish-ocher, with the following longitudinal markings: A series of 9 punctured lines, 5 of which reach the apex; a buff line on the sutural margin and a similar, although redder, space between the fourth and fifth and the sixth and seventh punctured lines. Each space is interrupted several times before it finally meets with the others near the apex of the elytron. The elytra bear near the base |⌈ and ⌉| and on the apical half ⌈⌉ on the left and right, respectively. There is also a broad, black, uninterrupted band on the external margin, confluent with a similar one on the prothorax. In the male these black characters are more or less confluent. The apices of the wing covers are emarginate at the suture; the pygidium is truncate, with the sides gradually converging; the median portion of the ventral surface of the thorax and abdomen is black and glossy, with numerous spine-bearing punctures; the posterior margins of the meso- and metathorax are deeply notched; the fore coxæ or first leg joints are almost contiguous, the interspace having a transverse suture. An elytron of the female is shown by Plate XI, fig. 5.

The *legs* are stout and moderately long; the femora are slightly swollen at their apices; the tibiæ of the middle legs are somewhat shorter than those of the fore and hind ones, and all are longitudinally ribbed with spine-bearing tubercles of minute size. The apices of all tibiæ bear a large tooth and two stout bristles. The 4-jointed tarsi are covered sparsely above and densely beneath with golden-brown hairs. The bidentate claws are long and graceful. These beetles have no constant external evidences of sex differentiation. (See Pl. XI, fig. 2, showing hind legs.)

Remedies and preventives.—The same preventive measures and remedies apply in combating this insect as are recommended in the case of the Asiatic palm weevil. The damage done by them is not by any means so extensive as that due to the other insect, but, nevertheless, it should, if possible, be prevented or stopped, as the tree is finally killed by the summation of the attacks of the various insects which it harbors.

THE FOUR-SPOTTED COCONUT WEEVIL.

The length of this beetle, exclusive of the snout, is 5 millimeters, and the width is 1.5 millimeters. It was found in the dead or decayed heart or the undeveloped leaves of a small 3-year-old coconut tree during a search for the rhinoceros beetle. It attacks only dead trees of a very small size and is met with only in coconuts. In addition to the adult beetles, the larvæ and the pupæ were secured in numbers. Plate IX, figs. 1 and 2, shows the exit holes of the adults and the work of the larvæ in the interior of the tree.

***Egg.*—The egg of this species is not known. It is probable that the beetles deposit their eggs on the sticky sides of their galleries in the trees, though close search failed to reveal them; but, as these places are also occupied by many**

other refuse-destroying insects and mites, it is probable that few of the shells would remain after the young grubs had emerged. Doubtless some of the mites feed upon the eggs themselves and in this way serve to limit the number of the beetles.

Larva.—The larva is of about the same general shape as that of the shot-hole coconut weevil, but it is more slender in proportion to its length. The length is 7 millimeters and the width 2.5 millimeters. The color is a pale-cream, the head being somewhat darker and the mouth parts dark-brown. The head bears numerous scattered, golden-brown bristles. The posterior margin of the labium is rounded, with a sharp angle at the median line. The ventral surface of the first thoracic segment is microscopically and densely spinose-tuberculated, as are also certain transverse areas on the ventrum and dorsum of the middle abdominal segments. The spiracles are extremely minute, somewhat slender, and pyriform, with lines radiating from the central slit to the margin. The upper, posterior surface of the last abdominal segment is slightly excavated, with 10 rather fine bristles; it is of a golden-brown on its margin. The larvæ feed in well-defined burrows or galleries slightly isolated from each other.

Pupa.—The length of the pupa is 5.5 millimeters and the width at the middle 1.75 millimeters. It is cream-colored and in general shape like the pupa of the shot-hole coconut weevil. Golden-brown spinose hairs are arranged as follows: Two pairs, very small, on the rostrum above the antennæ, 2 larger ones in front of the eyes, 2 still larger ones on the top of the head back of the eyes, 8 pairs symmetrically on the prothorax, 7 on the meso- and metathorax, respectively, 2 on each abdominal segment from the first to the sixth, 1 on the seventh, 1 at the base of the pygydium, and 1 on the ventral apical margin of the last abdominal segment, pointing downward. Each femur is provided at the outer apical angle with a single erect spinose hair. The spiracles are hardly visible.

Adult.—The general color of the beetle is dark-brown, with rufous patches. The head is globular and strongly punctured. The eyes are black and broadly crescent-shaped, contiguous beneath the head, but separated above by a narrow shallow sulcus at the base of the rostrum. The rostrum is slender, subcylindrical, slightly swollen laterally at the base above the insertion of the antennæ, and coarsely punctured, each of these punctures as well as all others upon the surface of the body containing a single club-shaped hair or bristle. (Pl. X, fig. 6.) The mouth parts are extremely minute. The mandibles are tridentate, and when closed are almost completely hidden within the mouth cavity. A narrow longitudinal sulcus is situated on each side of the mentum, into which fit the maxillary palpi. The antennæ are 8-jointed and of the same length as the rostrum; the first joint is slightly shorter than the other seven, the last is double the diameter of the preceding one and its distal half is silvery-pubescent with sensitive hairs.

The thorax is truncately conical and its anterior and posterior margins straight and parallel, the former having a narrow, smooth collar, back of which are numerous setigerous pits or punctures. It is coarsely and deeply punctured, with an indistinct rufous spot on each side. The scutellum is subtriangular and excavated at its middle. Each elytron is marked by 2 reddish-subquadrate spots, one at the base and the other beyond the middle, and is traversed longitudinally by 5 very finely punctured ridges or carinæ. Between every 2 carinæ there is a double row of very regular, coarse, deep punctures. The apex of each elytron is rather sharply rounded, the pygidium is subtriangular, and its sides and median line are carinated and rather densely setose, the setæ springing from fine punctures. It is very easily depressed; in some specimens it forms an angle of nearly 90° with the remainder of the abdomen. The legs are stout, moderately long, and the pairs about equidistant from each other and from the 2 extremities of the body, roughly dividing the latter into 4 subequal sections, if the rostrum

is excluded. The femora are swollen toward the apical angle and each has a slight constriction beneath and just in front of the apex. Each tibia has an apical claw and a tuft of pre-apical hairs beneath. The tarsi are all of equal length. The legs are conspicuously shiny in comparison with the rest of the body. Plate X, fig. 8, shows profile and fig. 7 an antenna of this beetle.

As is the case with nearly all weevils, these also feign death when annoyed. They conceal themselves under any available object and unless disturbed remain in one spot in their burrows for a long period.[4]

Remedies and preventives.—These beetles are found only in locations where others have preceded them and killed the trees; hence, they are not in any sense a menace to the healthy tree. Their description has here been given merely to call attention to all forms which may be encountered.

[4] It was hoped that the identifications of the shot-hole coconut weevil, the bonñgga weevil, and four-spotted coconut weevil would be received from Washington in time for insertion in this article, but as they have been delayed, it was thought best to publish the paper and give the identifications later.

[Part II of this paper will treat of the insects which live upon the leaves of the coconut, including two species of Lepidoptera and certain species of Coccidæ, of which there are several that make their homes upon the coconut. There will also be given a bibliography of the insects of the coconut.]

ILLUSTRATIONS.

PLATE I.

FIG. 1. Adults of *R. ferrugineus* Fabr. (About natural size.)
2. Head of larva. (X 5.)
3. Antenna. (X 12.)
4. Left mandible of female:
   (*a*) Profile and inner surface, showing condyle. (X 7.)
   (*b*) Interior view. (X 7.)
5. Profile of head and thorax of female. (X 1½.)
6. Profile of head and thorax of male. (X 1½.)

PLATE II.

Coconut showing results of attacks of *Oryctes rhinoceros* L. and *Rhynchophorus ferrugineus* Fabr.

PLATE III.

FIG. 1. Crown of coconut, showing inverted cone, in longitudinal section, eaten out by larvæ of *O. rhinoceros* and *R. ferrugineus* Fabr. (About one-seventh natural size.)
2. Adult of *O. rhinoceros* L. boring into petiole of coconut leaf. (One-half natural size.)

PLATE IV.

FIG. 1. Larva of *Oryctes rhinoceros* L.
2. Pupa of *Oryctes rhinoceros* L.
3. Adults, male and female, of *O. rhinoceros* L. (All about natural size.)

PLATE V.

FIGS. 1–4. Heart of coconut showing burrow made by adult of *O. rhinoceros*, and a female beetle working at center of tree. (One-half natural size.)

PLATE VI.

FIG. 1. Egg of *Rhynchophorus ferrugineus* Fabr.; magnified portion shown at 1 *b*.
2. Head of larva of same. (X 2½.)
3. Labium of larva of same. (X 3.)
4. Diagram of work of larvæ in base of coconut trunk, showing points of entrance, as at A.

PLATE VII.

FIG. 1. Larva of *Rhynchophorus ferrugineus* Fabr.
2. Pupa of *Rhynchophorus ferrugineus* Fabr.
3. Cocoon of *R. ferrugineus* Fabr., showing beetle partly emerged.
4. Cocoon of *Cyrtotrachelus* sp. (All about natural size.)

PLATE VIII.

FIG. 1. Adults of *R. ferrugineus* Fabr. (About natural size.)
2. Adults of *Rhynchophorus* sp. (About natural size.)
3. Work of larvæ of *R. ferrugineus* Fabr. in wood of coconut near roots. The larvæ entered from the lower left.

PLATE IX.

FIGS. 1, 2. Work of shot-hole coconut weevil in trunk of coconut, with exit holes of adults.

PLATE X. (Drawn by W. Schultze.)

FIG. 1. Larva of shot-hole coconut weevil. (X 3.)
2. Antenna of shot-hole coconut weevil. (X 3.)
3. Pupa of shot-hole coconut weevil.
4. Adult of shot-hole coconut weevil. (X 4.)
5. Adult of shot-hole coconut weevil, profile. (X 4.)
6. Adult of four-spotted coconut weevil. (X 8.)
7. Antenna of same. (X 36.)
8. Profile of same. (X 8.)

PLATE XI. (Drawn by W. Schultze.)

FIG. 1. Larva of *Cyrtotrachelus* sp. (X 5.)
2. Hind legs of adult *Cyrtotrachelus* sp. (X 10.)
3. Pupa of *Cyrtotrachelus* sp. (X 5.)
4. Adult male of *Cyrtotrachelus* sp. (X 5.)
5. Elytron of female of *Cyrtotrachelus* sp. (X 5.)
6. Profile of male of *Cyrtotrachelus* sp. (X 5.)

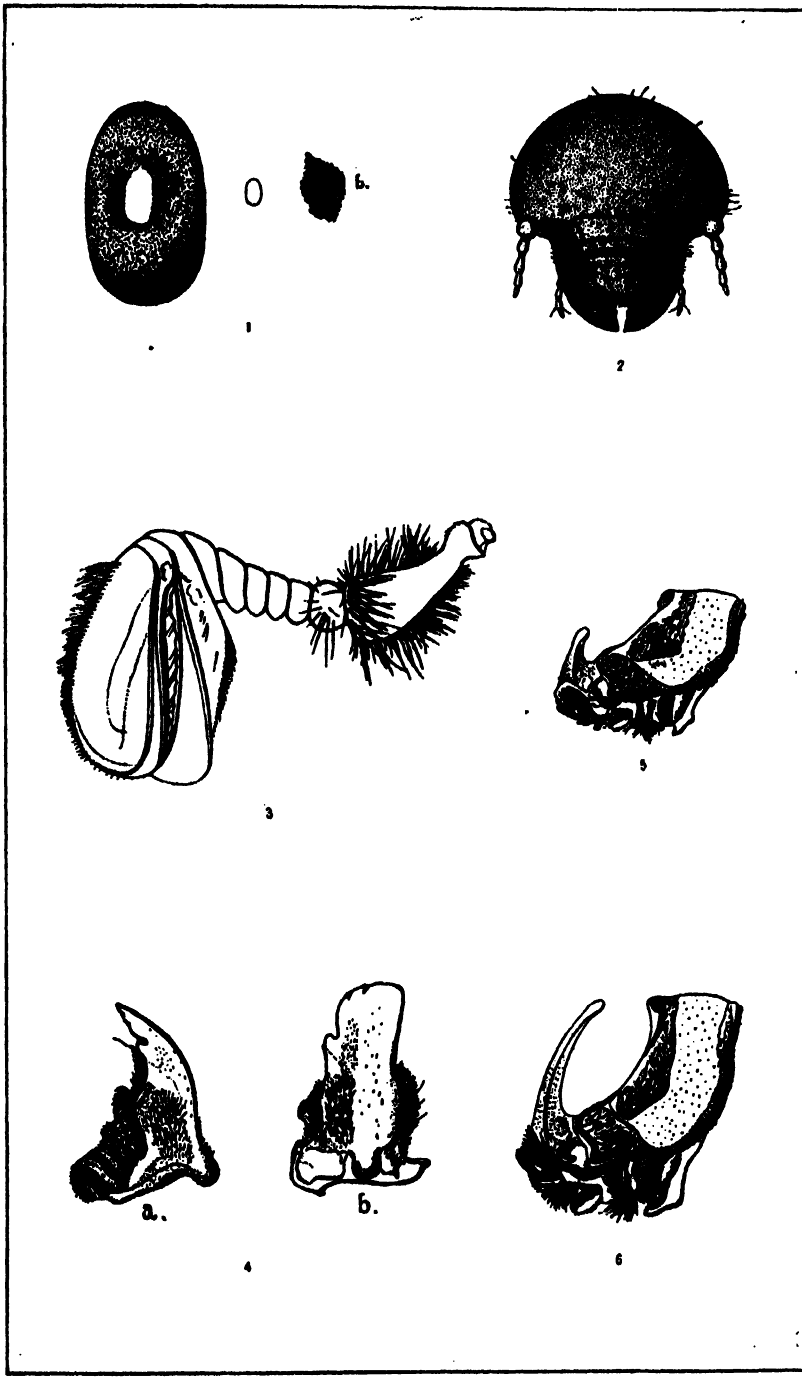
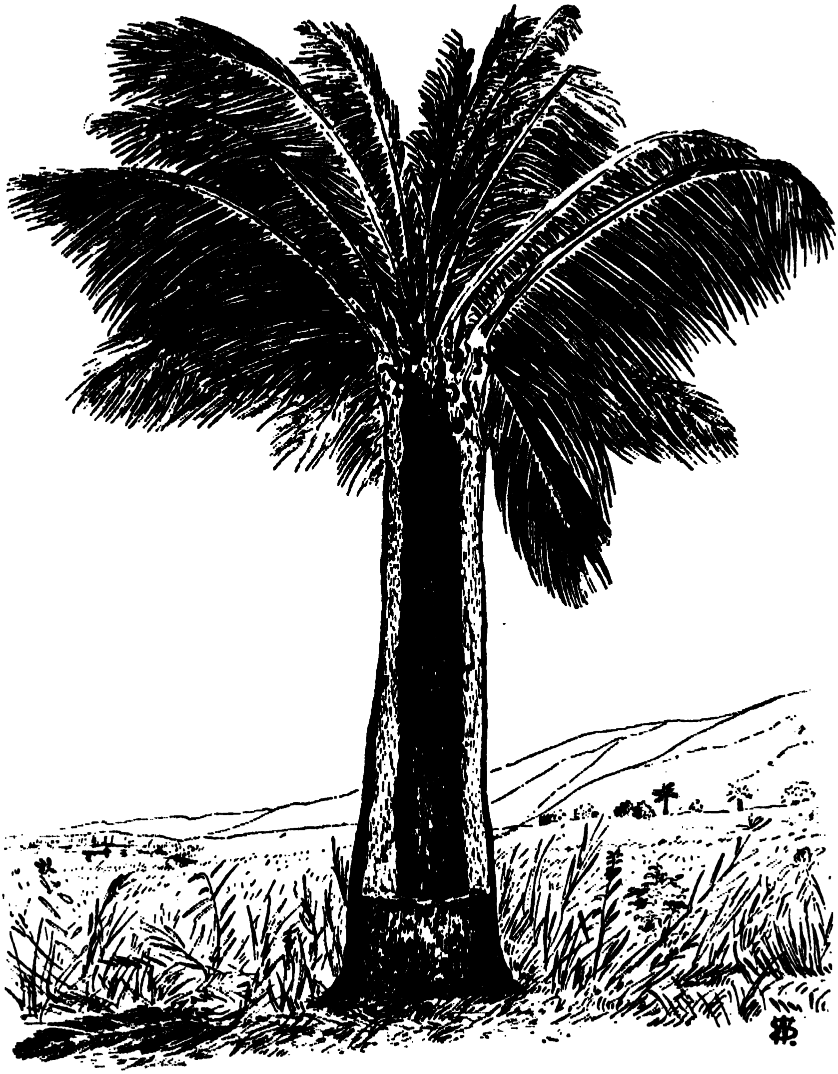


PLATE I.



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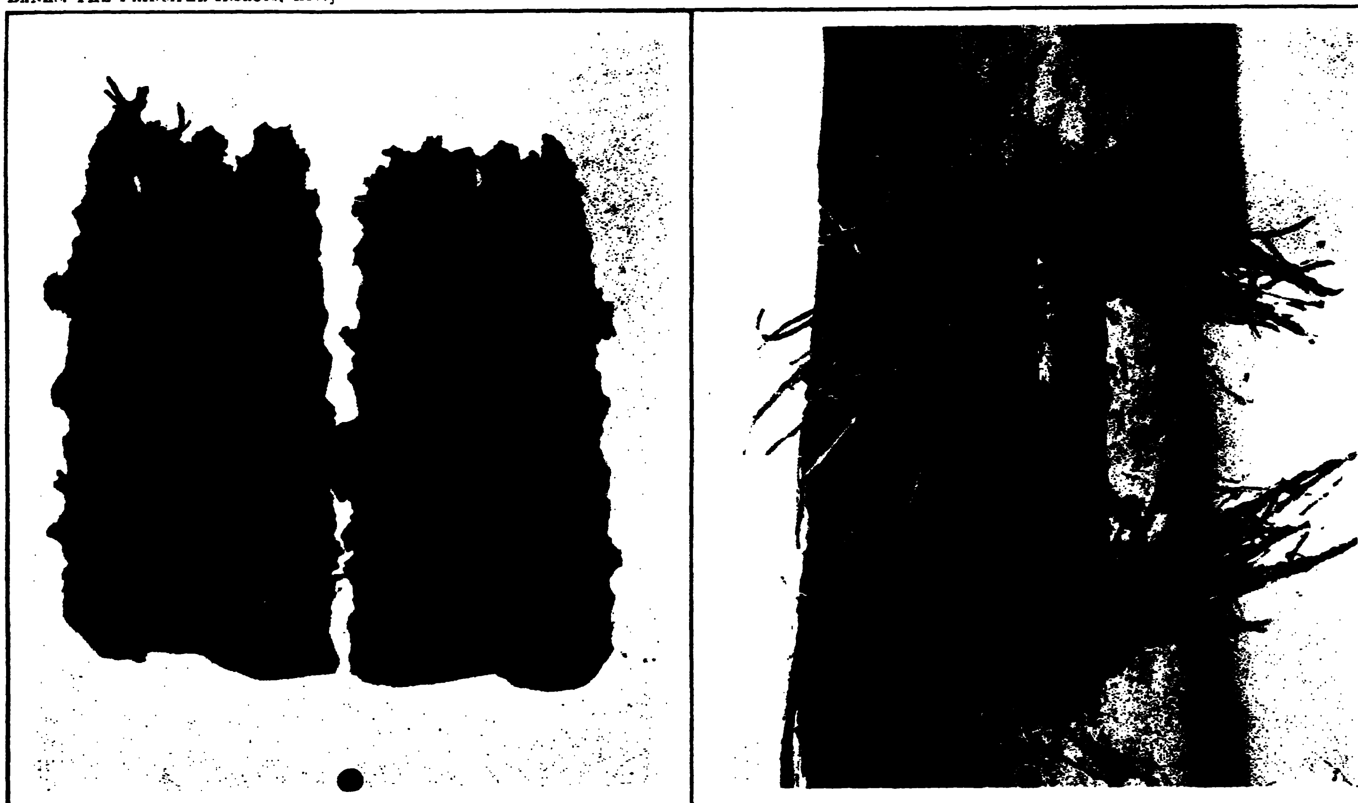
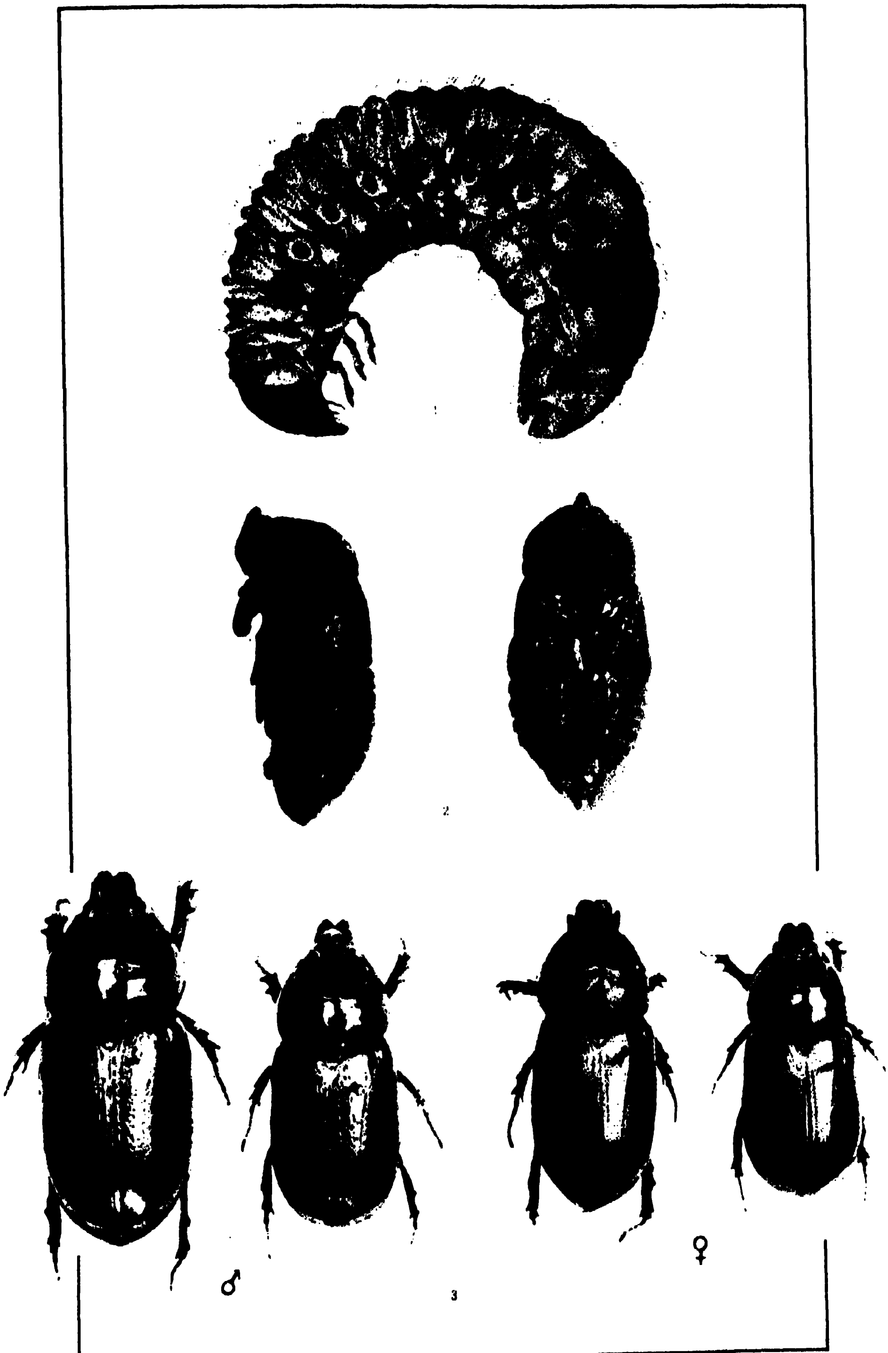


PLATE III.



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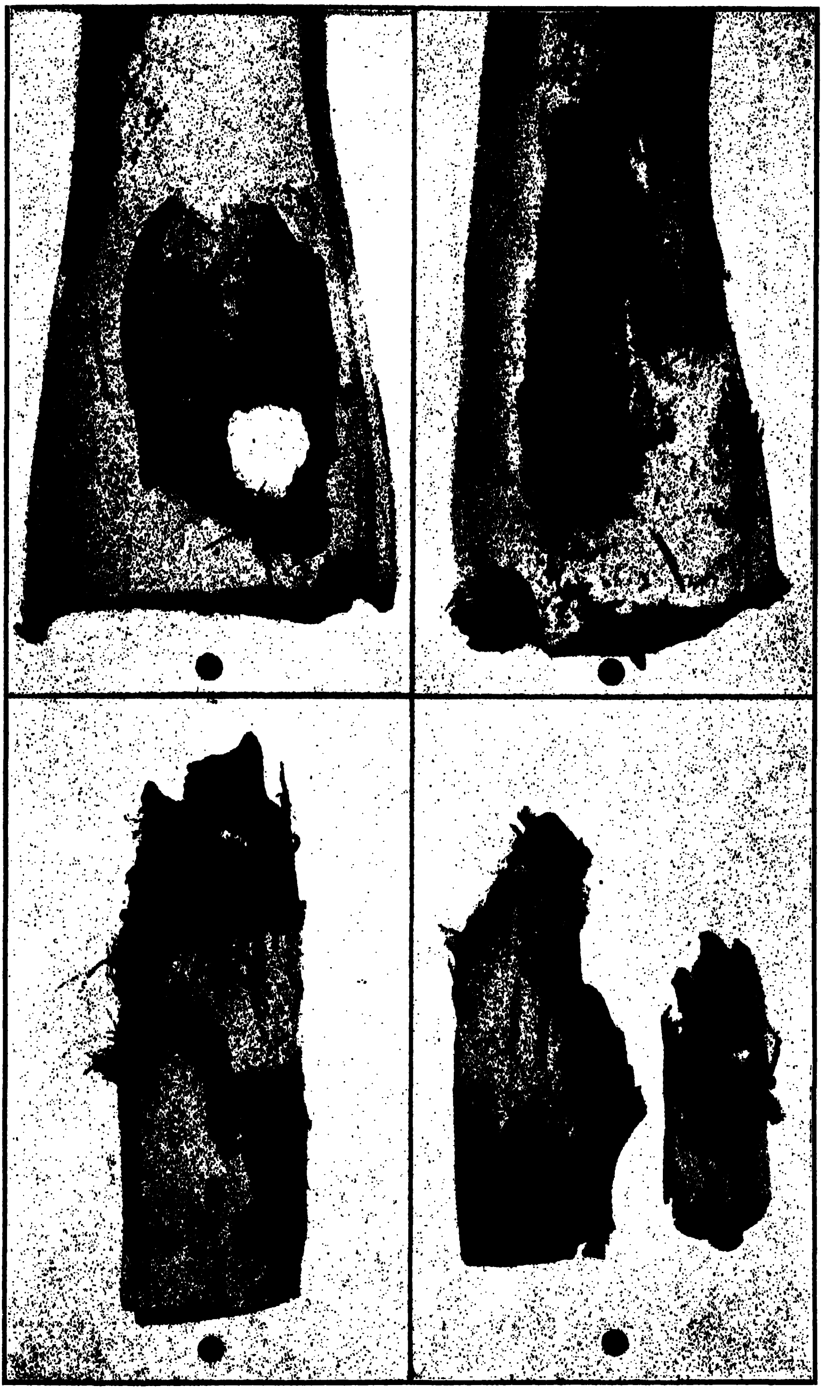
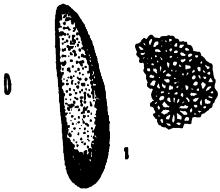


PLATE V.



b.

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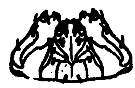

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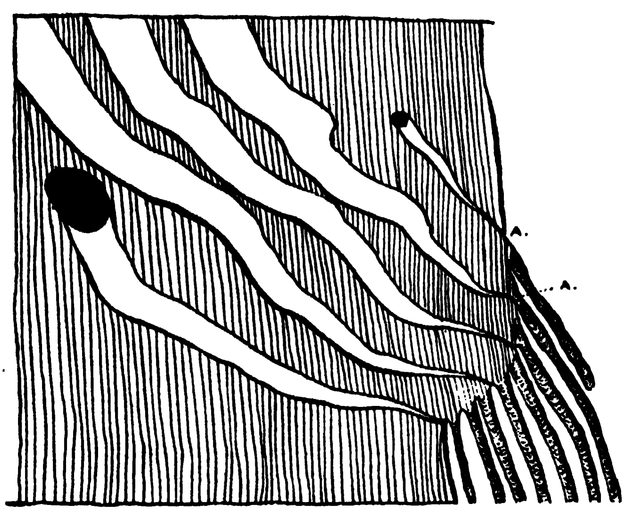

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PLATE VI.

PLATE VII.

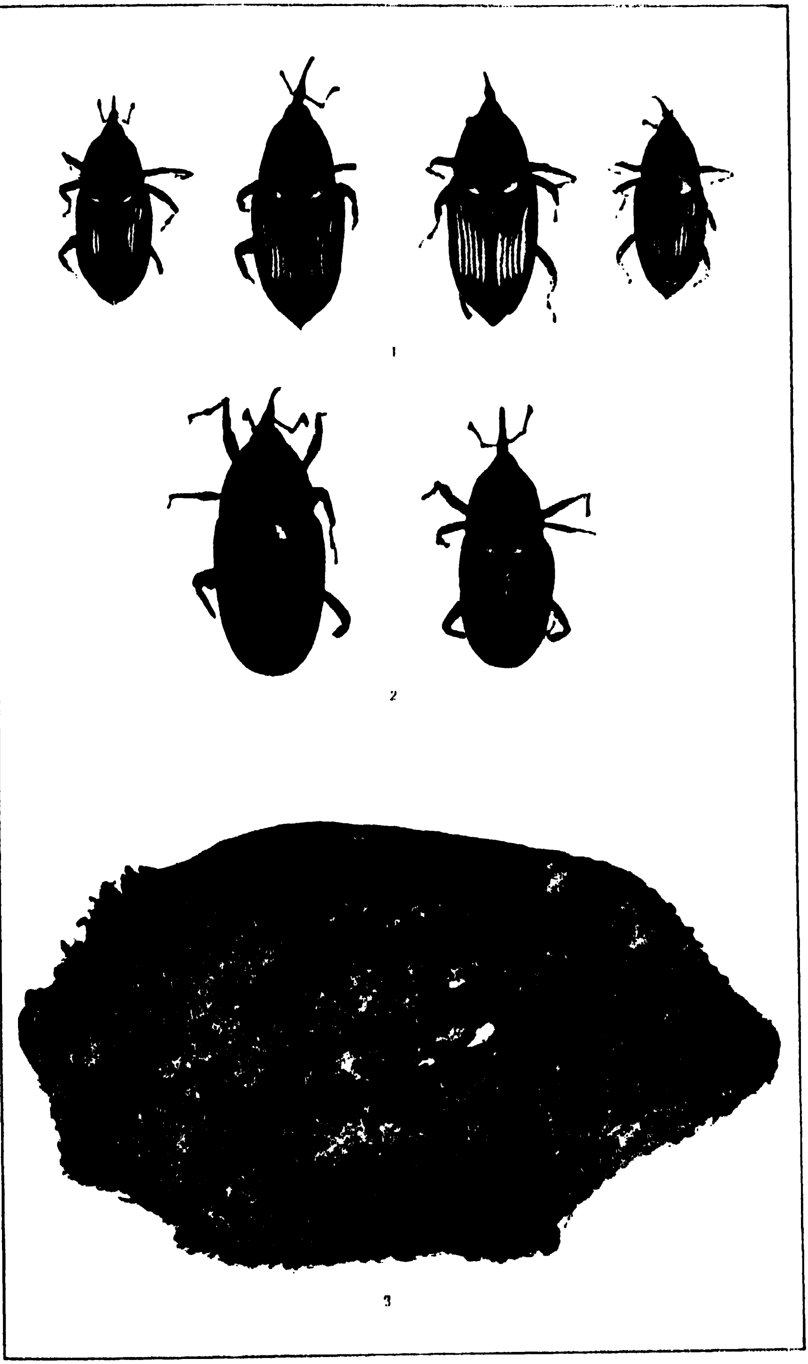

PLATE VIII.

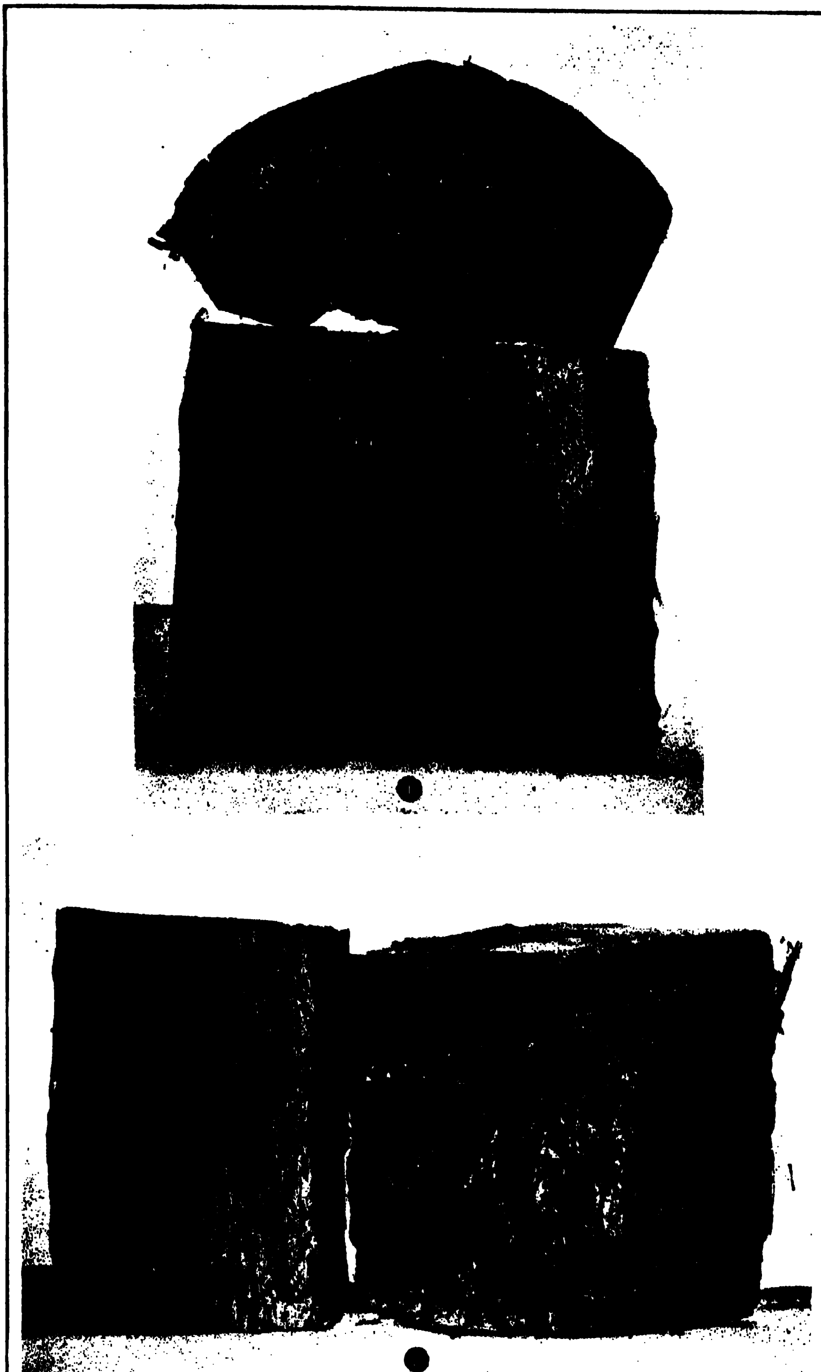

PLATE IX.

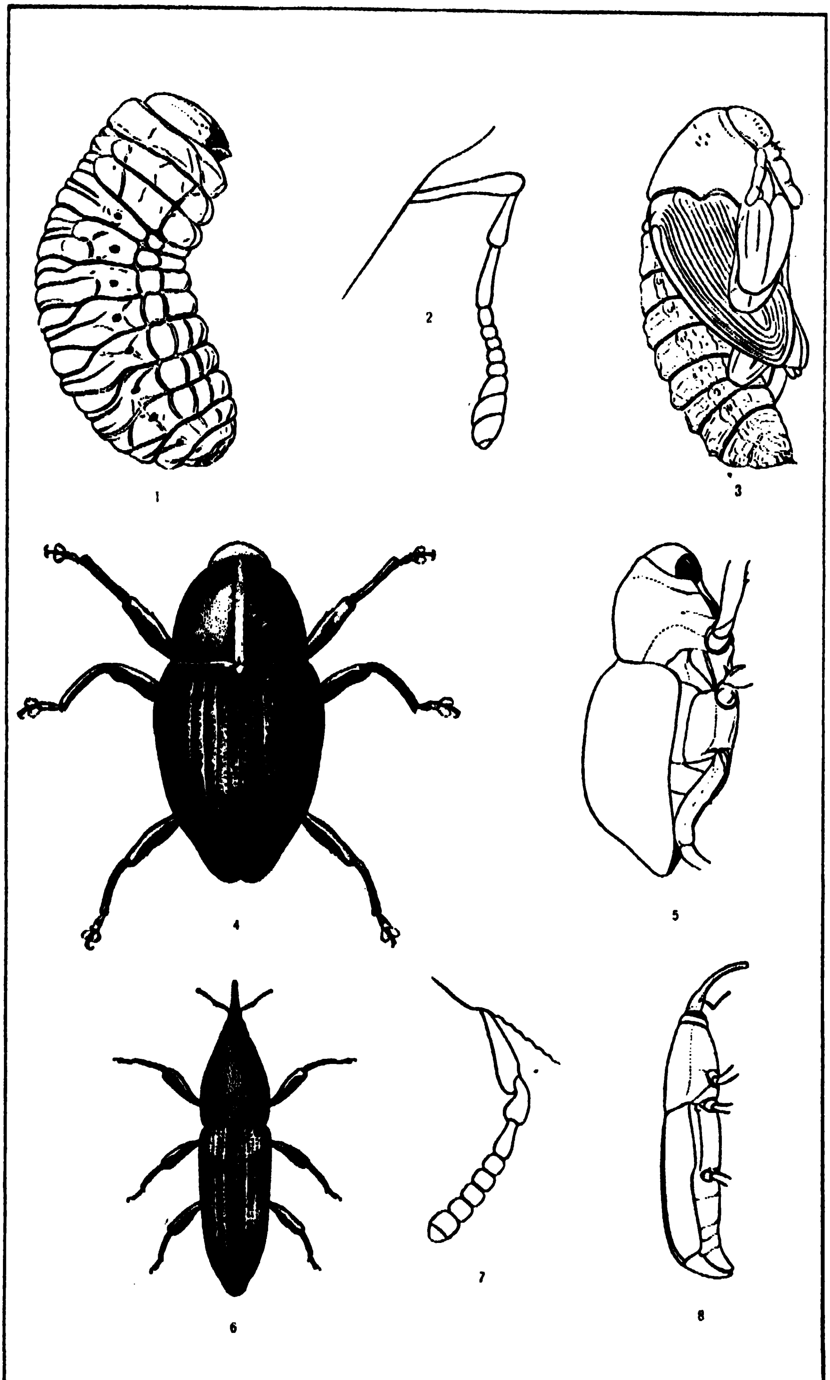

PLATE X.

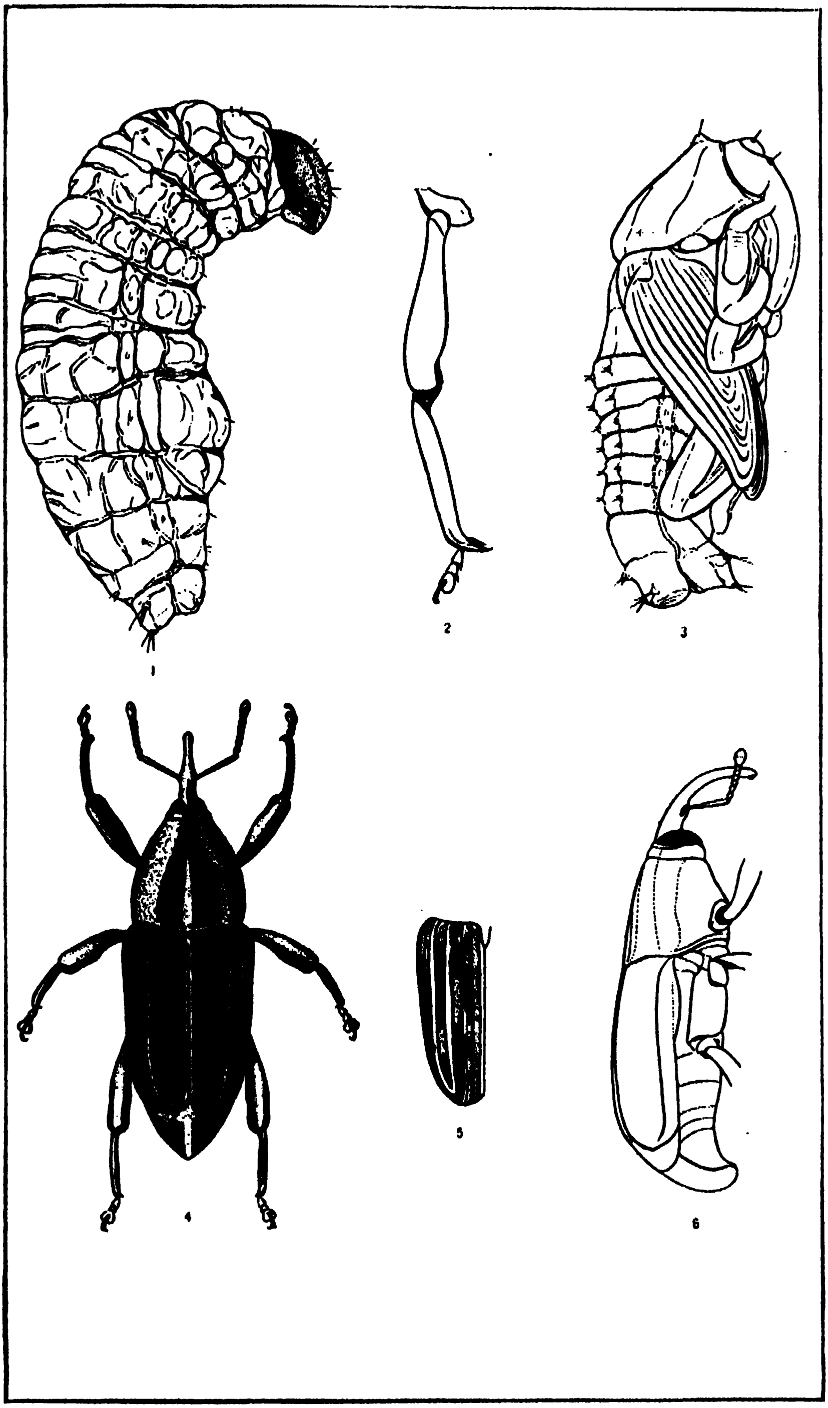

PLATE XI.

BERI-BERI IN THE JAPANESE ARMY DURING THE LATE WAR: THE KAKKE COCCUS OF OKATA-KOKUBO.

(A PRELIMINARY REPORT.)

By MAXIMILIAN HERZOG.

(*From the Biological Laboratory, Bureau of Science.*)

The affection most commonly known in European and American literature as beri-beri, and universally designated in Japan as *kakke*, is a disease generally confined to tropical and subtropical zones and particularly prevalent in certain parts of Asia, namely, Japan, China, the Malay Peninsula, the Dutch colonies, and the Philippine Islands. Especially in former years, beri-beri was exceedingly widespread in Japan, and it is from this country that most of our exact original knowledge concerning the disease has come. In this connection the work of Baelz, Scheube, M. Miura, K. Miura, and Yamagiwa, among others, should always be favorably remembered. During the late Russo-Japanese war much discussion was carried on, both in the medical and in the secular press, concerning the excellent management by the Japanese army medical corps of the sanitary affairs of the Japanese army; indeed, as it appears at present, the latter to a great extent succeeded in limiting any serious outbreak of typhoid, typhus, dysentery, or scorbutus, and in preventing entirely the appearance of cholera and plague in the rank and file of their fighting bodies. However, reports came to Manila during the second year of the war (1905) to the effect that a very large number of cases of beri-beri, occurring among soldiers returned incapacitated from the front because of this affection, were accumulating in the military reserve hospitals of Japan. As will appear from the official figures to be given below, this was indeed the case; and it was true to so great an extent that probably no outside observer, during the progress of the war, had any conception of it. It may here be stated that the total number of cases of beri-beri which developed in the Japanese army during this period is to be placed at a minimum of from 75,000 to 80,000.

However, it would certainly be doing a great injustice to blame the Japanese army medical corps for this great epidemic. There are certain points which must be considered when one is dealing with an occurrence such as this. Beri-beri is a disease the etiology of which is ill understood at the present time. We neither know its specific cause nor by what

route the specific poison, whatever it may be, enters the body. It is a well-known fact that, given certain conditions, no ordinary means of hygiene and sanitation will prevent the outbreak and the spreading of beri-beri. Such conditions are encountered in a particularly susceptible race, particularly when they are crowded together, and in certain localities. When these factors are present, nothing less than the abandonment of the locality which is apparently infected and the dispersion of the susceptible individuals will prevent the continual spreading of the disease. Of course, such measures as these could not very well be resorted to in the case of an army in the field and in the presence of the enemy. To repeat, given certain environments and certain compulsory conditions, *kakke*, in the present state of our knowledge, can not be considered to be a preventable disease.

The author was ordered to Japan to study beri-beri some time after the report concerning the presence of the disease in the military hospitals of Japan, and the information that two Japanese military surgeons claimed to have discovered its specific organism, had reached Manila. He was provided with letters of introduction from Mr. Goro Narita, Japanese consul at Manila, and he is greatly indebted to this gentleman as well as to Mr. Ishi, director of the Bureau of Commerce, Foreign Office, Tokyo, for securing permission, through the Japanese War Office, for him to pursue his studies in the military reserve hospitals at Hiroshima and Tokyo. He was also greatly assisted in the preliminary steps taken in arranging this matter by Mr. Griscom, American minister at the court of Tokyo; Mr. Huntington Wilson, first secretary of the legation, and Mr. Charles B. Harris, American consul at Nagasaki.

The author left Manila on August 15, 1905, reached Nagasaki on August 20, and arrived in Hiroshima on the 24th of the same month. At this place, situated not far from the Inland Sea, there had been a number of military reserve hospitals in operation during the war, with an aggregate capacity of 12,000 or more patients, the Japanese having transported all of their sick and wounded who could at all be moved, from the front to the home country. Hiroshima was one of the principal places for the reception of such invalids, and at that place, during the greater part of the war, there had been in operation one hospital devoted exclusively to the care of *kakke* patients. After the Japanese war office had given its permission for the pursuance of this work, the writer, through Colonel Onishi, chief medical executive officer at Hiroshima, was assigned to the *kakke* hospital to take up his studies under the direction of Surgeon Major Kokubo, who, before the outbreak of hostilities, had been professor of infectious diseases in the Military Medical School at Tokyo[1] and who was in charge of the hospital. In this institution of about 1,000 beds, there were remaining approximately 700 subacute or chronic cases of beri-beri. Connected with the hospital and situated in a separate building there was a small, though well-equipped laboratory for all ordinary bacteriologic work. Here

[1] This school was closed temporarily during the war, all of its teachers having been assigned to duty with the army.

the work in connection with the investigation of the etiology of beri-beri had been carried on by Dr. Kokubo, and here the author also worked for three weeks in isolating and examining, by Kokubo's method, the coccus which he and Okata maintained to be the specific micro-organism of beri-beri.

Kokubo had been most successful in isolating this coccus from the urine of beri-beri cases. His method was as follows: The ordinary, slightly alkaline agar medium was first melted in tubes and then poured into Petri dishes. After the agar in the plates was thoroughly hardened and dried, it was inoculated from the urine which had previously been collected in sterile receptacles, by making streaks over the surface of the media with a platinum loop. The Petri dishes were then placed in the incubator in an inverted position and examined at first after twenty-four hours and then successively from day to day. The author examined the urine from about thirty to forty cases of beri-beri and in eight was successful in obtaining a coccus which apparently was identical with the one which Kokubo believed to be the cause of the disease.

The agglutination test was regarded as the most significant feature in the identification of this coccus. Kokubo, by injecting rabbits, had prepared an antiserum which in the hanging-drop test, within one to two hours or even sooner, promptly agglutinated this particular organism in a dilution of 1–100. However, the author was not successful in obtaining this coccus from the blood of patients sick with beri-beri. He was never personally allowed to draw any blood from the sick soldiers; but Dr. Kokubo obtained some from several patients in his presence and inoculated a number of tubes with it. Dr. Kokubo's method of taking the blood was as follows:

An area on the back, over the trapezius muscle was cleansed antiseptically and then a puncture was made with a sterile, protected blood lancet. A fold of the skin on which the small punctured wound was present was then raised between the two fingers of the cleansed hand of the operator and firm pressure applied. A considerable amount of blood could thus be obtained for the purpose of inoculating a number of tubes and procuring cover-glass preparations.

It appears that this method is not free from objection, because of the fact that it may frequently, and in a number of cases will certainly, lead to an admixture of pressed-out lymph with the blood and to the danger of contamination by means of micro-organisms inhabiting the sweat or sebaceous glands. The collection of blood by direct puncture of the median cephalic vein was suggested to Professor Kokubo, but the surgeon in charge of the *kakke* hospital refused to employ this method on his soldier patients.

The writer spent his mornings in working in the laboratory of the hospital, whereas the afternoons were devoted to bedside studies which were made while accompanying Dr. Kokubo on his daily rounds, and the latter was kind enough successively to demonstrate all of the cases at that time

present in the hospital, the patients who were more seriously and more acutely affected being repeatedly seen and examined. It was impossible to see more than one post-mortem examination; this occurred in the service of Surgeon Lieutenant-Colonel Shimada.[2] Surgeon-General Okata was also present at this autopsy and he subsequently informed the writer that from the kidneys and the cerebro-spinal fluid he had succeeded in isolating from this case the coccus which Kokubo and he had previously obtained *inter vitam* from the urine and from the blood of *kakke* cases.

In September, 1905, Okata and Kokubo published their first preliminary report on their beri-beri investigation, it appearing in the *Journal of the Military Surgical Association,* printed in Japanese. Surgeon-General Okata, who is professor of bacteriology in the Military Medical School at Tokyo, was kind enough to furnish the writer with a copy.[3] The following extract includes all of its main features:

REPORT ON THE OKATA-KOKUBO BERI-BERI COCCUS.

On examining the blood of beri-beri patients we sometimes find cocci in microscopical preparations; these are occasionally met with intracorpuscularly and sometimes outside the corpuscles. They generally appear as diplococci, but are also seen as individuals. Occasionally they are observed in the form of staphylococci. These cocci do not stain uniformly, but show an uncolored slit in the center. They are not very numerous in general, there being only one or two observed in the field. These cocci have no capsule and are not motile.

For the purpose of obtaining cultures from beri-beri cases, the blood is collected as follows: (1) The region over the trapezius is cleaned with soap and water, (2) washed with bichloride solution, (3) with physiological salt solution, (4) with distilled water, (5) with sterile alcohol, and (6) finally with sterile water. The area is then punctured with a sterile lancet and the flowing blood is utilized to inoculate a number of tubes and to make some cover-glass preparations.

The number of patients examined by this method was 129. We had 65 cases in which both microscopical cover-glass examinations and cultures gave positive results. In 34 cases both were negative, in 11 cases the microscopical examination was positive and the cultures negative, and in 19 cases the microscopical examination was negative and the cultures were positive.

Staining.—All the aniline stains, color the coccus deeply. It is well stained with Loeffler's alkaline methylene blue, and still better by the method of Semenowicz and Marzinowsky[4] (a combination of the staining solutions of Ziehl and Loeffler).

Culture.—All ordinary agar media may be used. The most suitable temperature is 30° to 37° C. When the cultures are kept at this temperature a growth, visible to the naked eye, develops after eighteen hours. At room temperature (10° C.) a very slight growth is observed after three weeks. When bouillon tubes are inoculated and placed in the incubator, a slight turbidity shows itself after fifteen hours. After twenty-four hours a grayish-white sediment forms at the bottom

[2] In the Japanese military service, autopsies can be held only after special permission from the family of the dead person is received. This permission is only very rarely given.

[3] For a translation of this report the author is indebted to Mr. C. J. Arnell, of this Bureau.

[4] *Centralblatt für Bakteriologie* (1897), 21, 874.

and some of the growth may adhere to the sides of the vessel; but the fluid is perfectly clear. The appearance presented is similar to that found in cultures of the *Erysipelas streptococcus*. When a bouillon culture is shaken, the growth forms a stringy mass of grayish-white color; and the substance which arises from the bottom of the vessel is similar in appearance to that observed in bouillon cultures of *Spirillum rubrum*. The growth is quite sticky, and on being touched with the platinum wire it forms long strings, which are not easily separated. The organism does not ferment sugar. It grows in milk without coagulating the casein.

After four days in gelatine stick cultures, kept at 18° C., there appear, along the line of the puncture, minute, whitish granules, which afterwards become larger and confluent. In three weeks the growth assumes a yellowish color. There is no liquefaction.

On agar streak cultures there first develops a grayish-white growth, which in the course of time becomes yellowish. On agar tubes kept in the incubator, after twenty-four hours there develops a granular moist growth. If examined with a magnifying glass, the colonies are seen to be granular, the margin transparent, and the center dark-yellowish.

On glycerine agar, the transparent margin is more marked and the development of the coccus is similar to that in common agar cultures. The development on urine agar is similar.

On potato, a fine, light-yellow, dry deposit develops after twenty-four hours; this does not enlarge very markedly afterwards.

On blood serum, after eighteen hours in the incubator, a very luxuriant growth develops in the line of the streak; this is shining, grayish-white in color and moist. After further time the growth spreads out from the streak in all directions in the form of branches. (In culture media to which litmus has been added, no change of color occurs.)

Urine.—The morning urine of 34 patients was collected in sterile flasks. From these specimens culture media were inoculated. In 25 of these cases we succeeded in obtaining the coccus.

Fæces.—The fæces from 44 cases were collected in sterile vessels and diluted with physiologic salt solution. We were successful in isolating the coccus on agar in 15 of these cases.

Animal experiments.—Twenty-one rabbits were inoculated; of these, 3 died. The first, inoculated with human blood, died twenty days after inoculation; the second, inoculated from the spleen of a white mouse previously inoculated from a pure culture, died nine days after inoculation; and the third, inoculated with cultures, died eight days thereafter.

Seven guinea pigs were inoculated with human blood and with the heart's blood and spleens of white mice. None of these died.

Sixty-four white mice were inoculated, of which 17 died. Thirty-one of these were inoculated with human blood, of which two died; and 33 were inoculated from cultures, of which 15 died.

Methods.—The injection into rabbits consisted of one tube of blood and sterile salt solution into the veins. In guinea pigs the same amount was injected intraperitoneally or subcutaneously. In the case of the white mice, three-tenths of a tube (0.3 cubic centimeter?) was inoculated intraperitoneally, or else a platinum loopful of the pure blood was injected subcutaneously. One platinum loopful of agar culture mixed with sterile water was injected intraperitoneally into mice, or the same amount subcutaneously. The juice from the internal organs of animals dead of the disease was also injected.*

* The last part of the report is not clear. The translation here given is as literal as possible.

(In the description of the anatomical and histological changes in the animals which died from an injection of cultures of the *Kakke coccus*, the authors do not make any statement as to the condition of the peripheral nerves.)

After having seen, and studied, more or less, all of the cases of beri-beri in the hospital in charge of Surgeon-Major Kokubo, and after having obtained a number of cultures of the coccus, which, according to Okata and Kokubo, is the cause of this disease, the author left Hiroshima about the middle of September, 1905, and proceeded to Tokyo, where, during the war, five large military hospitals had been in operation. In two of these, namely, the Shibuya and Toyama hospitals, there were still present between 700 and 800 beri-beri patients. While visiting the Shibuya Hospital the author had the advantage of having the material demonstrated by Prof. M. Miura, senior professor of pathology in the Imperial University and one of the greatest Japanese authorities on beri-beri. Professor Miura had just returned from Manchuria, where he had been ordered during the latter part of the war by the Japanese Government, and was at this time attached to the Shibuya Hospital as chief consultant for beri-beri cases. Professor Miura is still an adherent of the theory that beri-beri is a disease introduced into the human organism by food. He attributed the greater prevalence of beri-beri among the Japanese soldiers in the field during the first year, as compared with the second one of the campaign, to the following facts:

During the first year almost all of the food for the Japanese army was imported into Manchuria from Japan; during the second one, on the contrary, the commissary department (transportation department) had developed a system of purchasing and transportation in Manchuria and the surrounding parts of the continent of Asia, which enabled it to obtain its food supplies from these places without depending upon importation from Japan. Professor Miura believes that the cases of beri-beri in the field were not due to infection from those which had come from Japan to Manchuria, but to the food supply exported from Japan to the army in the field.

The author afterwards visited Toyama Hospital, where he saw a large number of beri-beri cases. This is not only the largest hospital in Tokyo but in Japan, and perhaps in the world, as it accommodates 7,000 patients. The institution is situated on a very large tract of ground on the outskirts of Tokyo, this area having formerly been occupied by the Imperial Military School for Noncommissioned Officers. It now contains from 50 to 55 different hospital and administration buildings. While in Tokyo, the laboratory of Surgeon-General Okata in the Military Medical School was also visited, and Professor Okata very kindly furnished a number of stems of his *Kakke coccus*. The following statistics as to the occurrence of *kakke* in the Japanese army during the first year of the late

war were furnished to the author during the last days of his stay by Surgeon-General Koike, chief of the medical bureau of the war office:

[Thirty-seventh year of Meiji (1904) from the beginning of the war until the month of December.]

A table showing cases of beri-beri, both those returned from the field of war and those developed at home.

Date.	Cases returned from field.		Cases developed at home.	
	New cases.	Deaths.	New cases.	Deaths.
February			80	
March	10		107	
April	65		218	2
May	108		334	1
June	253	2	313	2
July	1,602	14	424	3
August	7,960	161	596	14
September	13,505	369	373	9
October	10,811	240	326	3
November	9,344	159	296	6
December	6,682	79	320	4
Total	50,340	1,024	3,387	44

Remarks.—(1) This table after further verification will probably show a few changes.

(2) This table shows the statistics of the prevalence of beri-beri, when it was at its height last year (year 37). Although since January of the present year there has been a great diminution in the disease, exact figures can not be given, as reports from all districts have not as yet been received.

(3) Special district, regimental, and classified military reports can not yet be prepared owing to the same reasons as above given.

The *kakke* material which the writer was able to study in Japan belonged to the hydropic and to the atrophic, dry variety of beri-beri. Acute, pernicious cases were not encountered, because, of course, these had to remain at the front, most of them probably dying there. The large beri-beri material concentrated during the recent war in the military reserve hospitals of Japan, as far as the clinical histories were concerned, fully confirmed the clinical descriptions of the disease which had previously emanated from Japan. Therefore, in this preliminary report, it is not desirable to enter more fully into the subject; but the histories of three cases which were kindly furnished to us by Surgeon-Major S. Kitamura, stationed at the Shibuya Hospital, are appended. It appears that careful histories were kept of all the cases in the military hospitals, surgical as well as medical, and the whole management of these hospitals appears to have been most excellent. All of the institutions which the writer saw, excepting a very few which were older and more permanent structures, were buildings which had been erected during the

war. They were of rather cheap frame construction, but were very practical in arrangement and scrupulously clean, being well adapted to the purposes for which they were intended. Every hospital had its laboratory facilities, and in several, complete X-ray and photographic outfits were seen.

The three histories referred to follow. One of them, No. II, is illustrated by a photograph, also kindly furnished by Dr. Kitamura.

Case No. I.

W. S., 22 years old; infantry. The patient comes from a healthy family, has always been perfectly well, and has never suffered from any disease. About the beginning of June, while with the army in Manchuria, he noticed, without being able to assign any special reason for it, a loss of appetite, anorexia, palpitation of the heart, and precordial anxiety. After a short time, paresthesia in the tips of the fingers and in the region of the thigh was noticed, then paresis of the thigh and pain in the calves manifested themselves. The patient entered the Tokyo military hospital at Shibuya on August 6, 1905.

Status praesens: He is a man of medium size, well developed, nutrition fair. Pulse 86, full and strong. No fever. Face somewhat puffed, but no true œdema. Tongue coated; skin dry. Pupillary reaction on both sides normal. Lungs: Examination negative. *Heart:* Upper boundary of the heart's area of dullness is found at the upper margin of the fourth rib, at the left, one finger's width externally to the left mamillary line, to the right, in the midsternal line. The first mitral sound is impure. The second pulmonary sound is accentuated. There is no epigastric pulsation. Arterial sound heard indistinctly. The upper margin of the liver dullness is at the upper margin of the fourth rib. Abdomen somewhat distended; epigastrium somewhat sensitive to pressure. Appetite good. Stools, one per day, small in quantity and soft in consistency.

Disturbances of motion and sensation.—Circular hypesthetic area around the mouth. Mouth can not be very firmly closed, and it is especially the upper lip which is distinctly paretic. (Such a condition around the mouth, the report says, is very rarely seen in *kakke.*) The entire upper extremities are hypesthetic. The senses of touch, temperature, and pressure are equally disturbed. The disturbances are more marked on the anterior than on the posterior surfaces, and more on the left side than on the right one. Both flexion and extension at the elbow and wrist are diminished. Flexion of the fingers is possible, but extension is very much disturbed. All the fingers are now in a flexed position, particularly the middle and the index finger.

From the umbilicus downward anteriorly and from the gluteal region downward posteriorly to the tips of the toes, there is hypesthesia. The latter is more marked on the interior surface of the thigh than on the exterior one. The power of flexion and extension at the knee joint is diminished, more on the left side than on the right one. The dorsal flexion of the feet is decreased; the plantar flexion, however, is normal. The muscles of the calf, quadriceps femoris and adductor femoris, and the muscles of the anterior side of the forearm, are sensitive to pressure. The patellar reflex, the reflex of the tendo achilles and that of the muscles of the hand are absent, as is also that of the anterior abdominal wall, of the cremaster, and plantaris; however, the adductor reflex is preserved.

Therapy: Magnesium sulfuricum, acidum hydrochloricum.

August 13: Condition of patient improved. Pulse 72. Appetite good. Three to four stools a day. Hands and feet somewhat cyanotic. Hypesthesia around the mouth is decreased. The motility of the lower extremities is improved.

August 21: The hypesthesia around the mouth has disappeared.. The mouth can be closed normally. The extension of the fingers is improved. The hypesthesia of the abdominal wall has almost entirely disappeared; that of the extremities is much improved. The motility in the knee joints is better. The feet and toes can now be moved in a normal manner. The appetite is good. Stools three times a day. The patient can now walk with the aid of a cane.

August 28: Hypesthesia is present only below the elbow and below the knee joint.

September 6: Pulse 72. The heart's dullness on the right side is found in the left sternal line and on the left side in the left mamillary line. Heart sounds pure. Second pulmonic is not accentuated. The extension of the fingers is still somewhat insufficient. The patient can now walk well with the aid of a cane; however, his gait is still a little unsteady.

Case No. II.

S. O., 23 years old; infantry. There is no hereditary taint. The patient has always been well and has never suffered from any severe disease. Some time before the beginning of November, 1904, he noticed a loss of appetite, palpitation of the heart, and œdema of the legs. He also had a pain in the calves, and it was noticed that the patellar reflex was absent. The heart's area of dullness was increased both toward the right and toward the left. Accentuation of the heart sounds.

November 22: Pulse 108. There is no fever. There is violent palpitation of the heart. The first mitral sound is impure, and the second pulmonary sound is accentuated. There is œdema over the whole body. The abdominal wall and the lower extremities are hypesthetic.

Therapy: Infusion of digitalis and *kali aceticum.*

December 10: Appetite is good. All the symptoms are improved.

December 13: The pulse is 100 and the temperature 36.7. There is one stool daily. The patellar reflex is absent.

January 27 (1905): The heart dullness at the right side is in the mid-sternal line. This patient came to the Shibuya Hospital with the foregoing history on February 8.

Status praesens: Poorly nourished, medium-sized man. Pulse 124; temperature 36.°8. Tongue clean; extremities very much emaciated. The first mitral sound is impure; the second pulmonic sound is accentuated. Appetite good. Stools once daily. The patellar reflex is absent on both sides. Paresthesia and hypesthesia on the lower extremities are confined to the feet. The muscles of the calf are sensitive to pressure. The dorsal flexion of the left hand is insufficient. All the fingers are in a flexed position and can not be extended. However, the hand and the fingers of the right side are almost normal. The motion at the knee joints is almost normal, but the feet and toes on both sides are totally immobile.

April 10: The left boundary of the heart's area of dullness is one finger's breadth inside the left mamillary line. At the apex the first sound is dull; the second pulmonic sound is accentuated. Pulse 120. Patellar reflex slightly present. The muscles of the calves are not so sensitive to pressure as previously. The motility of the hands and fingers is improved.

Therapy: Decoctum chinæ.

April 11: The thenar and hypothenar eminences are much emaciated.

April 22: The muscles of the legs show complete E. A. R. (reaction of degeneration).

April 26: Both feet are in an equino-varus position, in consequence of which they can not be used.

May 22: The muscles of the calf are no longer tender to pressure.

May 29: The patellar reflexes have become more distinct. Flexion and extension of the knee joints are much improved; however, the feet and the toes are still entirely immobile. The right hand and fingers are almost normal in motility.

June 24: The left foot has become somewhat mobile.

July 2: The left large toe has become somewhat mobile.

August 10: The muscles of the calf on both sides are somewhat indurated. On both plantar surfaces of the feet one feels some nodular hardenings, which are quite tender to pressure, in consequence of which the patient can not walk on his soles.

August 19: Both feet and both large toes have become mobile. In attempting passive dorsal flexure of the feet and toes, one encounters strong opposition, which is caused by the tendo achilles and the aponeurosis of the plantaris. The patient complains of pain along the flexor muscles of the leg.

August 31: The motility of the toes and feet is now better and the patient can walk with the aid of crutches.

Case No. 111.

T. I., 20 years old; in the military railway service. The patient comes from a healthy family and has always been well. About the beginning of August he had acute gastritis, lasting for three weeks. Shortly following it, there was noticed palpitation of the heart. The muscles of the calves were tender to pressure. There was hypesthesia of the legs. The patellar reflex had disappeared.

August 23: The patient was admitted to the hospital on this date. *Status praesens:* Medium-sized man, poorly nourished. Pulse 80, small and weak. The tongue is not coated. The voice is hoarse. The skin is dry. No œdema. The muscles and the subcutaneous tissue are somewhat diminished, especially at the extremities. *Heart:* The upper boundary of dullness is in the third intercostal space, extending to the right to the middle of the sternum and to the left to the left mamillary line. All the heart sounds are pure. The second pulmonic sound is somewhat accentuated. *Liver dullness:* The upper boundary is in the mamillary line at the upper margin of the sixth rib. The *abdomen* is moderately distended. The epigastrium and the hypogastrium are tender to pressure. A vibration can be distinctly felt at the left iliac region over the crural artery. The appetite is good. Stools twice daily. There are no dyspeptic symptoms. The hypesthetic areas are to be found only at the inner half of the feet. The right knee joint is slightly mobile; but the left one is entirely immobile. The dorsal flexion of the foot on the right side is fairly good; on the left side, however, it is disturbed. The motion in the toes is almost normal in excursion. However, the dorsal flexion of the large left toe is very insufficient. The muscles of the lower extremities are flabby and tender to pressure. This tenderness is especially marked in the adductor muscles of the thigh. The patellar reflex is completely abolished, as is also that of the tendo achilles, the abdominal wall, the cremaster and the plantaris. The triceps reflex is present.

August 30: The hypesthesia is now confined to one place, namely, the distal inner surface of the left foot. Both knee joints are somewhat immobile, particularly the left one. The dorsal flexion of the foot is improved, especially on the right side. The muscular pain is much lessened.

Therapy: Magnesium sulfuricum.

September 8: The dorsal flexion and the motility of the toes are much improved. The motility of the knee joints is now almost normal. The appetite is good. Stools once daily.

September 17: The extension at the knee joints is now almost normal in excursion.

The author, since his return from Japan, has been at work with the *Okata-Kokubo coccus* and has inoculated with it a number of monkeys and other animals. His results, as far as concerns the production in these animals of a disease similar to beri-beri in man, have not been encouraging. However, it is altogether too early to permit of any definite statements. These and other experiments will be fully dealt with in a future publication.

In concluding this preliminary report, it is my agreeable duty to express my sincerest thanks to those Japanese colleagues who have so liberally and willingly assisted me in the study of the beri-beri material to which I had access during my stay in Japan, and I wish particularly to thank Surgeons-General Koike and Okata, Colonel Onishi, Majors Kokubo, Shimada, Tanaka, Shimose, Hirai, and Kitamura, of the army, and Professors M. Miura, K. Miura, Kitasato, Shiga, and Doi, of the Imperial University and the Government Institute for Infectious Diseases, and also Captain Pershing, military attaché of the American legation, Tokyo.

ILLUSTRATIONS.

FIG. 1. A Japanese soldier at the Hiroshima Kakke Hospital. Atrophic, dry beri-beri; great atrophy of the muscles of the legs; contraction, with pes equinovarus. Author's original photograph.

2. Japanese soldier, Shibuya Hospital at Tokyo. Condition same as in No. 1. Photograph by Surgeon-Major Kitamura.

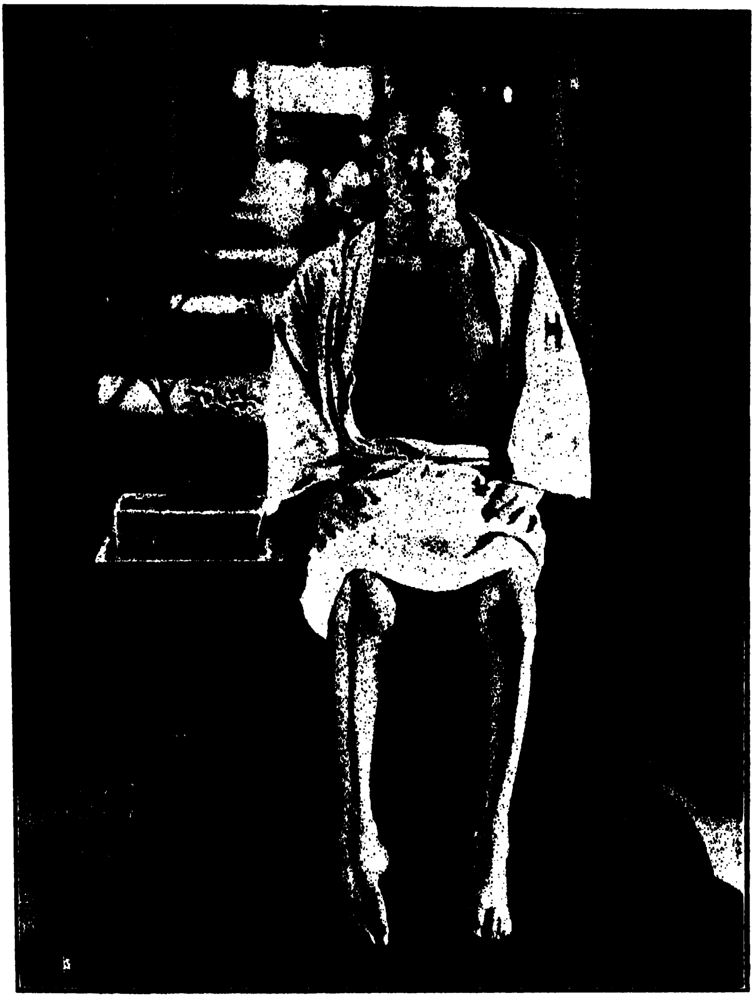

FIG. 1.

FIG. 2.

VACCINATION AGAINST PLAGUE.

By Richard P. Strong.
(*From the Biological Laboratory, Bureau of Science.*)

Although the question of protective inoculation against plague has received considerable attention during the past few years and prophylactics for the disease have been recommended by Haffkine,[1] the German Plague Commission [2] (Pfeiffer and Dieudonné), Lustig and Galeotti,[3] Terni and Bandi,[4] Shiga,[5] Besredka,[6] and Gosio,[7] apparently no successful experiments have been made on human *vaccination* against the malady—i. e., protective inoculation in which the living attenuated pest bacillus has been employed.[8] It is true that in the eighteenth century some desultory attempts were made to secure immunity in man by exposing the individual to direct infection. In 1755 the Hungarian physician, Weszpremi, suggested the artificial inoculation of the pest poison in a manner similar to that which, at that time, was practiced against smallpox (*variolation*), hoping in this way to produce a mild form of the infection. In 1781, Samoilowitz, a Russian physician, inoculated himself with plague pus, suffered a mild attack of the disease, and so became immune. Therefore, he recommended that a lint compress previously saturated with the pus from a plague bubo be bound upon the arm of the person to be immunized. The skin of the individual was not to be abraded. Other observers attempted similar experiments; but many of these resulted disastrously; thus, Cerutti performed such inoculations on six persons, five of whom died of plague. Because of these results this method of immunization obviously was soon abandoned and has not since been employed. Up

[1] *British Medical Journal* (1897), part 1, 1401. Also *Lancet* (1899), 1695.

[2] *Bericht der deutschen Pest Komission; Arb. a. d. Kais Ges. Amt.* (1899), 16, 306.

[3] *Deutsche med. Woch.* (1897), 23, 227, 289.

[4] *Deutsche med. Woch.* (1900), 26, 463. Also *Rev. d. Hyg.*, Paris (1900), 22, 62.

[5] *Bericht über die Pest in Kobe und Osaka,* Tokyo (1900), 54.

[6] *Ann. Institut. Past.* (1902) 16, 918. Also *Ibid*, July (1905), 19, 479.

[7] *Ztschr. f. Hyg.* (1905), 50, 519.

[8] Kolle and Otto have called attention to the fact that the term "vaccine" is more correctly employed in the sense in which it was primarily used by Jenner and Pasteur, and should not be applied to forms of protective inoculation in which the killed organisms or their extracts are used.

to the present time no further experiments of importance with the living organism, either in a virulent or an attenuated condition, have been made on human beings, but a number of this nature have been performed on animals.

The German Plague Commission [9] (Gaffky, Pfeiffer, and Dieudonné) called attention to the fact that an ape (*Macacus radiatus*) which had been inoculated with the living pest organism and which after many days' illness had recovered, was found to be fully immune about five weeks subsequent to the first inoculation, since, at that time, it resisted the injection of an entire oese of a virulent pest culture. For the purpose of attenuating the pest bacillus, agar cultures suspended in bouillon were exposed to a temperature of 51° C. during different periods of time.

First experiment.—The culture was heated for two hours at 51° C. and afterwards proved to be sterile. A monkey, inoculated with one-fifth oese of this culture, acquired no immunity, succumbing to an injection of one oese of the virulent pest organism made twelve days later.

Second experiment.—The culture was heated for one hour at the same temperature, but in this instance all of the bacilli were not killed. A monkey inoculated with one-fifth oese of this culture also showed no subsequent immunity.

Third experiment.—The organism was heated for only one-half hour at 51° C. In this instance many of the bacilli were also not killed, and the monkey inoculated with one-fifth oese of this culture died of pest four days after this primary injection.

In the foregoing observations, as was demonstrated by further experiments, the pest bacillus was not attenuated, but was either entirely killed or had retained its full virulence. In the second experiment, while the living organisms were still present in the vaccine, their number was obviously too small to produce the desired immunity or even to give rise to any decided reaction in the animal. This was conclusively shown to be the case by the following observation, in which a two-day agar culture was suspended in 5 cubic centimeters of bouillon and then further diluted with saline solution, so that ape A received 1 cubic centimeter in a dilution of 1 to 100,000; ape B, 1 cubic centimeter in a dilution of 1 to 10,000, and ape C, 1 cubic centimeter in one of 1 to 1,000, subcutaneously. No apparent reaction occurred from these injections and only ape C showed any traces of immunity, this animal remaining alive for nine days following the injection of 1 oese of the virulent pest bacillus (although it succumbed after this period), while the other two animals died on the third day after such an inoculation.

In another series of experiments, the commission attempted to obtain an attenuation of the organism by its exposure to the action of carbolic acid. However, no loss of virulence resulted by this method, the animals injected with the cultures which had been so treated all succumbing to pest infection. On account of the difficulties encountered in the attenuation of the plague bacillus or in obtaining cultures already attenuated,

[9] *Loc. cit.*, 303.

the commission abandoned the idea of employing the living organism for the purpose of obtaining immunity against the disease.

Albrecht and Gohn[10] (the Austrian Plague Commission) performed experiments with attenuated plague cultures on eight guinea pigs and twenty-seven rats. Of the guinea pigs inoculated, either subcutaneously or intraperitoneally, with the living organism of reduced virulence, and later repeatedly reinoculated with increasing doses of a virulent culture, five finally died and three remained alive. The immunity was found still to be present in some of the animals seven months after the vaccination. It is not altogether clear why repeated and increasing amounts of the more virulent organism were injected in testing the immunity of the guinea pigs, unless the authors felt uncertain of the exact virulence of the culture which they used or of the method they employed.

Kolle has recently criticised some of this experimental work more in detail, referring particularly to the fact that the animals were, at least in some cases, reinoculated with the virulent culture at too short a time after the vaccination, for the results of the experiments to be conclusive, since in some instances they might have been suffering from chronic pest at the time of the reinoculation. Besides this, the quantity of the bacteria injected in testing the immunity of some of the animals was very large. Twenty-one of the twenty-seven rats which Albrecht and Gohn vaccinated remained alive after reinoculation with the virulent organism. Only one experiment was performed upon a monkey; in this instance the animal was apparently immunized successfully, but it finally died of tuberculosis. Albrecht and Gohn conclude that immunity can be obtained in animals by employing the living pest bacillus, but that this process must be carried on in a careful manner in order obtain a fair degree of protection. From their experiments they were unable to decide whether the immunity caused by the injection of the killed pest bacillus was fully as great as that which resulted from the inoculation of the living organisms.

Yersin and Carré[11] also performed experiments upon the immunization of rats with attenuated strains of the plague bacillus. They finally obtained a culture of such diminished virulence that only one-fifth of the animals vaccinated with this organism succumbed to the effects of the injection. A series of twenty-five rats was inoculated with this culture. Three of these died from the effects of the vaccination and about three weeks later the remaining twenty-two animals were inoculated with the virulent pest organism, after which only one succumbed. A second series of twenty rats was inoculated with the same bacillus. Ten of these, which survived the vaccination, were later inoculated with the virulent

[10] *Denkschrift d. math.-naturw. Klasse d. Kaiserl. Akad. Wien* (1898 and 1900), **66**, 807.

[11] *Congrès International de Médecine, Section de Médecine et Chirugie Militaires. Sous-section Coloniale*, Paris (1904), 54.

pest culture, when all proved to be immune. Only two control animals were inoculated in each of these series. This number was hardly sufficient to render the experiments conclusive. The authors do not state the size of the dose, the virulence of the organism used in testing the immunity, and the method of inoculation. When this same attenuated bacillus was allowed to grow continuously on artificial media during forty to fifty days it was claimed that its virulence was greatly decreased, since of thirty rats inoculated with such a culture, none died. Later, on testing fourteen of these animals with the virulent pest organism, five succumbed. Seven apes were inoculated with another plague culture which, when injected into rats, killed from 40 to 50 per cent of these animals. None of the apes died from the effects of the vaccination. Apparently only two of these animals were subsequently tested for their immunity; these developed localized buboes, but recovered. Since the single ape used for control purposes also did not die we can draw almost no conclusions from these experiments. The amount of the organism employed in the vaccination or in the testing of the immunity is not stated. Yersin apparently was inoculated with the most attenuated culture (fifteen days old), but the size of the dose used in the vaccination is not given. Only very slight symptoms developed. At the same time, ten rats were injected with this culture but none died. The immunity of these animals had not been tested at the time the paper of these authors appeared. Although these experiments were reported in 1900, I have been unable to find any further reference made to them since that date either by Yersin or his colleagues.

No extensive or convincing experiments in regard to the value of the employment of the living attenuated cultures in the immunization even of animals against plague had apparently been undertaken until Kolle investigated this subject.

In 1902 and 1903 Kolle and Otto[12] inoculated eighteen guinea pigs subcutaneously with an attenuated culture of the pest bacillus. The organism was an old laboratory culture in which the reduction of the virulence had, in some unknown manner, taken place during its growth on artificial media. Buboes, which later discharged and healed and in the pus from which a few bacilli were present, developed in the animals, but they showed no other evidence of sickness and subsequently entirely recovered. The animals were reinoculated two, three, and eight months later with one-twentieth to one-fiftieth oese of a pest culture (of which one one-hundredth oese represented the fatal dose for a normal guinea pig). Seven of the animals remained alive.

In a large series of rats immunized by various methods, the loss from inoculation, with the living attenuated cultures, was 2.3 per cent; with the killed agar cultures, 33.3 per cent; with Haffkine's prophylactic, 38.5

[12] *Ztschr. f. Hyg.* (1903), 45, 512.

per cent; and with Lustig's prophylactic, 12 per cent. After reinoculation (for the purpose of testing the immunity of the rats), 45 per cent of those inoculated with the attenuated living organism, 21.9 per cent of those which had received the killed agar cultures, 22.2 per cent of those injected with Haffkine's prophylactic, and only 12 per cent of those which were given Lustig's preparation remained alive.

However, Kolle and Otto's further experiments on guinea pigs are more interesting and conclusive; for, as these authors have remarked, a preparation which is recommended and used for the control of plague in human beings should develop a pronounced protective effect on the guinea pig, the animal which is the most susceptible to plague. Among fifty-nine guinea pigs which they immunized with other attenuated living cultures, thirteen died and two were killed for control purposes. The remaining forty-four (75 per cent) were tested for their immunity. All of these had received but a single injection of the weakened culture; however, upon their reinoculation three, four, and eight months after this vaccination, twenty-eight (63.6 per cent) remained alive after the injection of the virulent organism. Six other guinea pigs received somewhat larger doses of a less attenuated culture, and at the same time an injection of serum; five survived the vaccination and one died. The former, upon reinoculation with the virulent organism, later all proved to be immune. Including the loss during vaccination, there finally remained alive, after testing the immunity, 50.8 per cent of the whole series.

Attempts were made to immunize twenty-six guinea pigs with *killed* agar cultures, amounts as large as from one-half to one entire agar culture being injected subcutaneously. During the process of immunization, four of the animals died; of the remaining twenty-two, only two (7.7 per cent of the whole) proved to be immune on subsequent testing. Hardly more favorable results were obtained in the experiments in which *killed bouillon cultures* were employed. Twenty animals were inoculated with Haffkine's prophylatic; two of these died during immunization and of the remaining eighteen, only two (10 per cent) remained alive after reinoculation with the virulent organism. The method of immunization, in which Haffkine's prophylactic was first injected and later followed by the introduction of the attenuated living cultures, did not give as favorable results as did the living avirulent organism alone.

In December of the past year (1904) Kolle and Otto[13] in further detail reported upon the immunization of guinea pigs with the attenuated pest bacillus. Among thirty-four of these animals immunized with such a culture (*Maassen V*), of which none died during the process of immunization, twenty-one were reinoculated with a virulent organism one to four months after their vaccination, and of this number sixteen (76 per cent) remained alive, and five died. Nine other guinea pigs were inoculated with the avirulent culture, and at the same time with plague

[13] *Ztschr. f. Hyg.*, December (1904), **48**, 399.

immune serum; all proved to be immune upon reinoculation with the virulent pest bacillus. The organism with which these vaccination experiments were performed possessed so little virulence that from two to three living, agar slant cultures, when injected into a guinea pig of 250 grams' weight, did not cause the death of the animal. In the experiments of Kolle and Otto on the vaccination of monkeys, almost all of the animals died from the effect of the attenuated culture or succumbed to intercurrent disease.

Other experiments on guinea pigs, in which repeated inoculations of the *killed* cultures of the plague organism were employed, were also performed. The animals were first injected with one, then with 1.5, and finally with two killed agar cultures, or with 1, 1.5, and 3 cubic centimeters of Haffkine's prophylactic. In the process of immunizing twenty guinea pigs, six of the animals died from the effects of such large doses of the killed bacteria. The immunity of the remaining fourteen, six weeks after the last injection, was tested with the living virulent plague bacillus, when only one animal remained alive and proved to be immune.

Therefore, Kolle emphasizes the fact that, if such large and repeated doses of the killed pest organism fail to immunize such small animals as guinea pigs, it seems unreasonable, from such a method, to expect very favorable results in man, particularly since in the latter case the amount of the bacteria inoculated is so much smaller in proportion to the body weight. It is to be noted that in immunizing the guinea pigs similar or larger doses of killed organism were employed than have been recommended for protective inoculation in human beings.

It is not my purpose here further to enter into the discussion of the protective value of the different prophylactics recommended for the immunization of man against plague. I had already concluded from animal experiments, as well as from the fact that a number of persons who had received several injections of Haffkine's prophylactic later sickened and died with plague that *the killed pest organism* constituted for man a far from satisfactory protective against this disease. On the other hand, the experiments which Kolle and his pupils had performed on guinea pigs seemed so conclusive in regard to the value of *the living attenuated cultures* in the immunization of these animals that I felt convinced that, if these cultures were of so low a virulence, or could be further so attenuated as to warrant their use in man, a higher degree of immunity could almost certainly be obtained with them than by the employment of the *killed* bouillon or agar cultures.

Accordingly, when Professor Kolle, after some correspondence on the subject, kindly offered me cultures of these attenuated pest organisms which had been employed in his experiments on guinea pigs, for use in the vaccination of human beings, I decided to carry on this work. My experiments in vaccination in man and animals have been performed with three attenuated strains of the pest bacillus, *Maassen All* and *Maassen V*

of Kolle and an old Manila culture which had been grown continuously upon artificial media for three years and whose virulence has been still further reduced by artificial means. The attenuation of this last culture was further brought about by growing the bacillus at a temperature of from 41° to 43° C. in flasks of alcoholic bouillon for three weeks at a time, as recommended by Otto. Cultures from these flasks were then inoculated on agar for many generations, a fresh generation being made every day for several weeks, and the organisms always cultivated at the same high temperature. Beginning with 0.05 cubic centimeter of absolute alcohol and 50 cubic centimeters of bouillon, the amount was gradually increased in successive cultures up to 5 cubic centimeters in 50 of bouillon. Before making inoculations in man, the action of the attenuated culture was of course carefully tested in animals.

In the present paper it is merely my desire to call attention to the fact that vaccination in man can with safety be performed with attenuated cultures of the living plague organism, and therefore only the human inoculations undertaken with one strain of this bacillus will be referred to.[14] The organism in question (*Ma. V*) possesses so little virulence that in a series of twelve guinea pigs and thirty monkeys inoculated with from one to two entire agar slant cultures, not one succumbed from the effects of the inoculation.[15] It was with this culture that the first experiments were performed in human beings. Since I believed that the guinea pig is an equally if not even a more susceptible organism than man to the pathogenic action of the plague bacillus, it was presumed that if this animal could invariably withstand the action of such large amounts as two whole agar slant cultures of the organism, much smaller quantities could be inoculated into human beings with safety, and indeed, before performing the experiments on man, I felt thoroughly convinced of this fact; nevertheless, the human inoculations were performed as carefully and with as much deliberation as possible.

The first injections were carried on upon prisoners under sentence of death; in the first case one-hundredth oese of the attenuated culture was inoculated subcutaneously without any noticeable effect. After ten days, ten other individuals were inoculated with the same dose, in order to demonstrate that no special natural immunity against the plague organism had been existent in the first instance. In this manner the amount of living organisms given was gradually increased, a single person being first inoculated with the larger dose and then, after it had been observed that no unfavorable effects occurred, from five to ten other

[14] The results of these experiments were communicated in a paper read before the Manila Medical Society at its meeting on November 6, 1905.

[15] But one of the animals of the series perished. Monkey No. 1299 died about twelve hours after inoculation with the avirulent pest organism, of a staphylococcus and streptococcus pyemia which had existed prior to the inoculation. A large suppurating wound existed over the abdomen.

persons were also treated with the same amount of the vaccine. This method of procedure was adopted in order to minimize the danger of inoculating a very susceptible individual with a dose which might prove disastrous. It was argued that if ten persons selected at random withstood the inoculation of a certain amount of the organism without developing unfavorable symptoms, a single individual, also selected at random, could probably receive a slightly larger dose without great danger. In this manner as mentioned the dose was gradually increased until one whole agar slant was inoculated. No attempt has been made to inject a larger amount of the organism, since from experiments performed on animals it has been concluded that a sufficient immunity in man will probably result from an inoculation of this quantity. Up to the present time forty-two persons have been injected with this large dose (one twenty-four hour agar slant culture) of the living bacillus, and, although the inoculations which I include in this report were all performed more than two months ago and the individuals treated have been under constant surveillance, I have no accident to report.

Surprising as it may seem, the injection of these large amounts of the living plague organism have not given rise to any very severe reactions. A few hours after the inoculation, the temperature of the individual usually begins to rise. When the injection has been given in the morning the fever may, on the evening of the first day, reach 38.9° to 39.4° C. (102° to 103° F.), but rarely has it touched 40° C. (104° F.). On the following day, in none of the cases was the temperature above 38.9° C. (102° F.) and usually not above 37.8° C. or 38.3° C. (100° or 101° F.) and on the third one it generally was normal. Occasionally the cases showed a moderate leucocytosis after the large injections. The organisms were always suspended in 1 cubic centimeter of .085 saline solution and the inoculations were made deeply into the deltoid muscle. On the day after the vaccination there usually was distinct induration about the point of injection, with some soreness on pressure, but these symptoms subsided in one or two days. No suppuration ever occurred. A careful study of the blood serum has been made in twenty-nine of the human cases; agglutintative tests have been performed with the virulent plague organism and the anti-infectious power of the serum has been tested in rats. A detailed report of all the experimental work will appear in a future number of this JOURNAL.

It was interesting to observe the length of time during which these avirulent pest organisms remained alive in monkeys after subcutaneous inoculation, and for this purpose a series of ten animals was injected upon different days, the cultures being taken at periods of from one to twenty-four hours after the inoculation. The abdomen of the animal was first shaved, and the injection made subcutaneously. The skin was then carefully massaged until apparently the fluid was completely absorbed. At the time the culture was to be taken, the skin of the abdomen

was scrubbed several times with ether and alcohol and a small incision made with a sterile knife through the dermis. The cultures were then made from the drops of blood which oozed from the incised wound. Usually, when the injection is made beneath the skin of the abdomen, after a few hours an œdematous swelling, which may not entirely disappear for from twelve to twenty-four hours, appears near the point of inoculation.

The different series of cultures made from the animals have shown that six to eight hours from the time of inoculation the organisms are still very numerous in the tissues, after which time they gradually disappear, so that the cultures made twenty-four hours subsequent to the injections remain sterile. It seems probable that the more resistent organisms are those which remain alive the longest, and that there is here a true survival of the fittest. A trial is therefore being made to ascertain whether it is possible to increase the virulence of these attenuated pest strains by such a procedure. As soon as the cultures made from the blood of one animal develop, they are inoculated into another monkey, and so on through a long series. It seems possible that such a method may have certain advantages over that in which the organisms are inclosed in celloidin sacs and placed in the abdominal cavity of animals. Kolle and Otto have shown that it is very difficult or impossible to increase the pathogenesis of more virulent strains of pest bacilli [16] by repeated passages through guinea pigs; but this need not necessarily be true with more avirulent strains.[17]

It may be questioned whether the organism which has been employed in these human inoculations is really a strain of *Bacillus pestis*. Therefore, while it is not considered necessary in detail here to relate the immunity reactions, morphology, etc., of this organism, it may be stated that unquestioned proof of this lies in the fact that I have vaccinated both guinea pigs and large numbers of monkeys with this culture and have later shown them to possess high and undoubted pest immunity by subsequently inoculating them with large amounts of virulent plague bacilli. Indeed, with no other method of inoculation have I been able to obtain such favorable results as with this pest *vaccine*.

Allusion has been made in other places to the similarity between certain of the immunity reactions of plague and of rinderpest. The present method of human vaccination against plague may be compared with that first recommended by Robert Koch in the immunization of cattle against rinderpest. Koch used the bile of animals which had died of this disease and in which *perhaps* the living attenuated organism of rinderpest exists. Doubtless a higher immunity in man against plague could be obtained were a more virulent culture of the pest bacillus

[16] Those which kill the animal.

[17] Those which are not capable of causing the death of the animal, even in large amounts.

injected and at the same time a dose of antiplague serum inoculated,[18] but there undoubtedly would be a mortality by such a method, just as in rinderpest there is always a certain fatality among cattle when the virulent blood and antirinderpestic serum are injected simultaneously, and therefore such a method of immunization, though valuable for animals, can not be recommended for man. Moreover, it is questionable whether any higher immunity in human beings than that which can be obtained from the attenuated, harmless culture is usually necessary.

In publishing for the first time the results of my vaccinations in human beings against plague, I wish to sound a note of warning against the employment of other strains of living pest bacilli for this purpose. Inoculations should not be made in man, unless the investigator can guarantee the organism with which he is working to be of sufficient attenuation to be no longer dangerous for human beings. Strains of the bacillus which invariably no longer kill guinea pigs in doses of two entire forty-eight hour agar slant cultures are probably safe in very small amounts for human beings. Unless excessive precautions are taken in inoculations of living plague bacilli, disastrous results will surely follow. It seems probable that by pursuing proper methods for sufficient periods of time all strains of pest bacilli perhaps can be attenuated sufficiently to be safe for the purposes of human inoculation; although in some instances it may take several years to bring about such a result. I believe that the avirulent pest culture with which these experiments were performed is at present hardly more dangerous for inoculation in man than is attenuated vaccine taken from human beings suffering with smallpox.

In concluding this preliminary report I wish to express my thanks to Professor Kolle for two of the avirulent pest cultures and again to call attention to the fact that it was the careful and extensive work on this subject which has come from his laboratory during the past two years which convinced me of the value of the living attenuated plague cultures in immunization and which caused me to undertake this further study of the question in man and animals.[19]

[18] My experiments have convinced me that in pest (as in cholera) unquestionably a higher immunity in animals can be obtained with the more virulent organism than with the less virulent one. The pest bacillus therefore may differ from the typhoid organism in this respect, since Wasserman has shown that this phenomenon does not necessarily result with the latter bacterium.

[19] While this article was in press there reached Manila the *Centralblatt für Bakteriologie* [(1905), **39**, 610] containing a report of the experiments of Hueppe and Kikuchi on immunization of animals against plague by means of "aggressin" obtained according to the method of Bail [*Archiv. f. Hyg.* (1905), **52**, 272]. The value of immunization with plague "aggressin" prepared after the method of Wassermann [Wassermann, and Citron, *Deutsche Medizinische Wochenschrift* (1905), **31**, 1101] as compared with that of the living attenuated plague organism will be discussed in the more complete report.

PHILIPPINE WOOD OILS.

By A. M. Clover.
(*From the Chemical Laboratory, Bureau of Science.*)

In a study of tropical forest products there are encountered as exudations from trees a great variety of substances, all of which may appropriately be termed resins. They appear and are collected in different physical conditions which are modified by the rapidity with which they issue from the tree and the rate at which they dry or harden thereafter. The latter function is dependent upon the relative amounts of water, oil, and solids which are found in the resins and upon the chemical composition of the oil. Accordingly, many resins are encountered in the solid form and contain very little volatile matter, whereas others collect upon the tree in a plastic condition and still others harden so slowly that they are removed as fluids.

The members of the latter class which have been differentiated in a study of the resinous products of the Philippine Islands have shown among themselves a similarity in chemical composition and a likeness to other known products to such an extent that they may appropriately be placed in a class by themselves and designated as *wood oils* (a term sometimes applied to gurjun balsam). A *wood oil* is therefore a fluid resin of very slight "drying" power and containing a high percentage of volatile matter; the oily portion of this volatile matter sometimes is as much as 75 per cent of the total resin and consists entirely of bodies belonging to the sesquiterpene group. In no case has a low-boiling or terpene oil been observed in this class of products; but on the other hand the viscous resins nearly always contain terpenes and the relative amount of oil found in them seldom exceeds 25 per cent.

OIL OF SUPA.

The tree (*Sindora wallichii* Benth) yielding this oil is said to be widely distributed over the Islands. The sample examined was sent to the laboratory from the Province of Tayabas, where in certain localities it is reported to be used as an illuminant, but no information concerning its use in other parts of the Islands could be obtained. A freshly cut tree, so it is stated, yields about 10 liters of the product, to obtain which it is

necessary to make a cavity in the trunk. Botanical specimens of the tree from which the sample under consideration was taken accompanied it and they were identified by Mr. Merrill, of this Bureau, it being the species given above. The oil is quite mobile, perfectly homogeneous, light yellow in color, with a slight fluorescence and a feeble but characteristic odor.

Specific gravity, $\frac{30^\circ}{30^\circ} = 0.9202$. Optical rotation, —31°.3 (10 centimeters, 30°). When cooled below 20° it begins to deposit white, flaky crystals; these increase in amount as the temperature is lowered. The crystals are those of a hydrocarbon, melting at 63° to 64°, and occurring in the oil to the extent of a few per cent. The oil completely dissolves in all the ordinary solvents, excepting alcohol, which causes the separation of the white crystalline hydrocarbon already referred to. Oil of Supa takes up oxygen slowly from the air and finally hardens, several weeks being necessary for the drying of a thin film.

Volatile portion.—When Oil of Supa was subjected to steam distillation, a colorless oil was slowly carried over, the process being continued until most of the latter products had been removed. On allowing the distillate to stand until it was perfectly clear it showed a rotation of —21° (10 centimeters, 30°). It was impossible to obtain a constant-boiling product from the latter upon fractioning under diminished pressure. At 40 millimeters nearly all of the oil passed over between 143° and 149°, the residue apparently being as fluid as the distillate and indicating no polymerization. The distillate had a specific gravity of $\left(\frac{30^\circ}{30^\circ}\right)$ 0.9053 and when redistilled it boiled between 255° and 267° (760 millimeters) with practically no residue. In order to save time it was found to be more convenient to remove the volatile oil by direct distillation under reduced pressure. With a pressure of 40 millimeters the temperature of the distillate gradually rose to 170°, at which point nearly all of the volatile portion was removed, the total quantity in the receiver consisting of about 73 per cent of the original sample, and containing only a slight amount of water. The residue was light brown in color and became semisolid on cooling; it dissolved in all the ordinary solvents, excepting alcohol, which separated the solid hydrocarbon referred to above. The distillate was colorless and practically remained so on standing in a closed vessel. It had the feeble, but characteristic, odor possessed by the steam-distilled oil, and on refractioning at 40 millimeters it almost completely passed over within 7°. No low-boiling substance was noted, and after repeated distillation it was not found possible to obtain a much more constant-boiling product. The oil probably is a mixture of sesquiterpenes, as cadinene was proven to be present. The absence of bodies of an alcoholic nature was demonstrated by the fact that neither sodium nor phosphorus pentoxide in benzol have any action on the oil. On passing hydrochloric acid gas into a solution of the distillate in acetic

acid, cadinene hydrochloride, as described by Wallach[1] (melting point 117° to 118°), separates.

0.1878 gram substance gave 0.1920 gram AgCl.

Required for $C_{15}H_{24}$, 2 HCl		Found
	Per cent.	Per cent.
Cl.	25.6	25.3

The hydrobromide was also prepared and recrystallized from ligroin. Its melting point (118° to 125°) corresponds to that obtained by Wallach.

On saturating a solution of one part of the distillate in four parts of glacial acetic acid with hydrochloric acid gas at 5°, the hydrochloride soon separated in considerable quantity. The solution was kept saturated for several hours and then allowed to stand for a day. It was then cooled, filtered quickly with suction, and washed with alcohol. Twenty grams of the hydrochloride were obtained from 45 grams of the oil. To convert the hydrochloride into cadinene it was heated with excess of aniline at 150°; aniline and aniline hydrochloride were removed with dilute acid and the remaining product was distilled with steam and redistilled under diminished pressure. It boiled at 164° to 165° (38 millimeters). Not enough of the substance was at hand for a determination of its specific gravity. Rotation, —39° (5 centimeters, 30°). It will be noted that the treatment with hydrochloric acid gas only partially converted the oil into cadinene hydrochloride, but a separate determination of the total amount of hydrochloric acid which the oil takes up under the conditions of the above experiment demonstrated that if it be considered as composed of sesquiterpenes, then, for every molecule of the latter it contained two of the gas.

It is evident that cadinene constitutes a large portion of the distillate from Oil of Supa. As to the remaining constituents nothing definite can be said, for no other crystalline derivatives could be obtained from the oil, although many attempts were made. One experiment in this direction was especially interesting because it resulted in the conversion of the oil into a constant-boiling product.

Twenty grams of the distillate were added to 100 cubic centimeters of alcohol and 5 cubic centimeters of dilute sulphuric acid (1 to 5) and heated for seven hours on a water bath with reflux condenser. Afterwards the alcohol was almost entirely distilled and the residue thoroughly shaken with water, after which it was dried and distilled under reduced pressure. The distillate passed over within 5°, leaving a small amount of a tarry residue. On redistillation the product passed over completely at 161° to 163° (37 millimeters). At 760 millimeters it boiled from 271° to 274°. (Cadinene of Wallach, 274° to 275°.) Rotation, +10.2° (10 centimeters, 30°). Specific gravity, $\left(\frac{30°}{30°}\right)$=0.9176. The oil possesses a slight odor which resembles that of cadinene but which is quite distinct from that of the original Oil of Supa. To judge from the boiling point, it would appear to be almost pure cadinene; however, it is dextro-rotatory, whereas the

[1] *Ann. d. Chemie* (*Liebig*) (1887), **238**, 80.

cadinene prepared from the hydrochloride is strongly laevo-rotatory, and the amount of cadinene hydrochloride which can be obtained from it is no greater than that which can be separated from the original distillate.

The distillate from Oil of Supa absorbs oxygen when it is exposed to the air and it gradually becomes viscous; in a thin layer it slowly hardens; a piece of muslin saturated with the oil and exposed to the action of the air so as to avoid evaporation showed an increase in weight of over 10 per cent in three weeks. This absorption is accompanied by a slight darkening in color. When a current of air is drawn through the oil at 200°, oxygen is very rapidly absorbed and the product becomes viscous and dark colored. The optical rotation of the distillate, removed at ordinary pressure, was found to be only —5.4° (10 cubic centimeters, 30°), while that of the steam-distilled product under the same conditions is—21°. This lowering of the rotation is due to the heating to which the oil is subjected by the former process, it being ascertained that by continued heating at the boiling point, the optical activity is entirely destroyed.

A study of the effect of heat upon the viscosity of the oil demonstrated that very little if any change occurred when it was kept at 250° for ten hours; the specific gravity of the product increased by 0.002, but there was no noticeable alteration in the boiling point.

Non-volatile portion.—This consisted of about 27 per cent of the total and remained as a residue after the distillation of the lower boiling substances at 40 millimeters was completed; after this point, even under greatly diminished pressure, decomposition began. The residue in part consisted of a solid body which separated on the addition of alcohol. It was recrystallized from the latter solvent, filtered to as dry a condition as possible, and then completely desiccated *in vacuo*.

The crystals cling together and are flaky and soft, melting at 63° to 65°. Their boiling point is very high, but heating in a test tube over a free flame causes no decomposition. The body is unaffected by alcoholic potash and it does not evolve hydrogen when treated with melted sodium.

0.2680 gram substance gave 0.8394 gram CO_2 and 0.3602 gram H_2O.

0.1494 gram substance gave 0.4658 gram CO_2 and 0.2010 gram H_2O.

	Found	
	(1)	(2)
C	85.43	85.02
H	14.94	14.61
Total	100.37	99.63

It is evident from the analyses that the body is a hydrocarbon. In chloroform solution it does not add bromine and on warming with dilute potassium permanganate the latter is not decolorized. Substances of the same type have previously been observed in resinous products and essential oils.[2]

[2] Gildemeister und Hoffmann: *Die Aetherischen Oele*, 158.

The saponification number of the residue was found by the usual method to be 64; and under the same conditions the free acid value was 60. These numbers show that the amount of saponifiable matter in the residue is practically *nil*. On taking up the product in ligroin and extracting it with a 10 per cent solution of caustic potash nothing is removed. On digesting it with a small amount of alcohol, which dissolved all but the hydrocarbon already considered, the latter was shown to constitute 25 per cent of the residue and accordingly, about 6 per cent of the original Oil of Supa. The remaining portion, after the evaporation of the alcohol, had the consistency of a thick sirup. On treating this with alcoholic potash on the water bath for several hours it suffered a change and it was then largely soluble in a solution of fixed alkali, notwithstanding the fact that the saponification number indicated no alteration by this treatment.

BALAO: OIL OF APITONG.

Several products from different species of the genus *Dipterocarpus* are utilized by the natives of the Philippines, but in this paper only the discussion of the viscous, slowly drying fluid products which may appropriately be termed wood oils will be entered into. The most widely used are those from the species *grandiflorus* and *vernifluus*, the oil from the former being generally known as *Balao*, and the tree from which it is derived as *Apitong;* that from the later as *Malapaho*, the tree being termed *Panao*. Besides the above there are several other wood oils of the same class which are used to a smaller extent. All of these products are similar in composition and consist of a solid resin, of water, and of from 25 to 40 per cent of a volatile oil. From the information which has been obtained it appears that their chief use is in calking small boats and furnishing a protective varnish for wood. For these purposes they are generally mixed with some other solid resin or with lime. At times they are also said to be used for illuminating oils and for torches.

Balao, according to the reports of the Bureau of Forestry, is a product which is in common use in nearly all of the provinces of the Islands. It is secured by allowing the resin to collect in a cup-shaped cavity which is cut in the body of the tree. As the flow decreases, the cup is cleaned, or, if an insufficient supply has passed, the resin is ignited *in situ*, which operation greatly increases the rapidity of the flow, although the product obtained by this means is dark in color, the maximum yield per day from a tree probably being not more than 1 kilo. The freshly exuded resin is white in color, but on standing it soon darkens. When spread upon a surface it slowly hardens to a tough varnish. The samples of this resin which were examined were from three different provinces, there being no doubt as to the botanical identification of the tree. The resin, as it is obtained under ordinary conditions, is a viscous fluid, which is more or less colored according to the time during which it has stood and to the amount of dirt and bark which may have found their way into it. It is not homogeneous, as it contains a large proportion of a granular solid which is immiscible with the fluid, and which remains in suspension. It

possesses a feeble, but characteristic, odor which serves to distinguish it and its oil from other similar products. It appears to dissolve in all the ordinary solvents excepting alcohol, although observations as to solubility are unsatisfactory because of the suspended water which is always present and which does not separate. When the resin is mixed with sesquiterpene oil and the mixture is heated at 140° in an oil bath, some of the water is gradually driven off, although it is necessary to heat to a much higher temperature in order to remove all. A clear solution is finally formed, but on cooling the product, because of the separation of a gelatinous substance, becomes semisolid. Its behavior with fatty oils is precisely the same. A clear solution is formed on heating to expel water, but a precipitation occurs on cooling. This point is important, as it concerns the use of the product for the manufacture of varnishes.

Although Balao is fluid at ordinary temperatures, it hardens when it is treated with steam and it then becomes much too viscous for the latter to penetrate it. Therefore, it is impossible to remove more than a trace of oil by this method. The separation of the volatile products from the resin by distillation under reduced pressure is an impossibility because of an uncontrollable foaming at the beginning of the operation and subsequently on account of the fact that the partially dehydrated residue turns nearly to a solid at the temperature at which the oil passes over. When the resin is gradually heated in a distilling flask which is immersed in an oil bath, only a portion of the water is removed by the time the temperature of the bath has been increased to 200°. In order successfully to remove all of the latter it is necessary to apply a free flame, under which treatment the solid residue gradually melts, and the water, together with a considerable quantity of oil, distill. The continued formation of so much water at a comparatively high temperature, while, at the same time, a large amount of sesquiterpene is being carried over, seems to show that the former liquid is chemically bound in the original oil; however, the combination evidently is not very stable. There is no evidence that the water is formed by destructive distillation resulting from the superheating of the resin, as the distillate is nearly colorless and no odors to be referred to decomposition products are noticeable. Oil continues to distill over on continued heating after all of the water has been driven off; this oil is almost colorless at first, but, as the temperature is increased, the distillate gradually assumes a reddish-green color and at 270° (vapor) there is evidence of decomposition in the flask.

The process just described was the only one found to be practicable for the study of the resin, and accordingly it was applied to the several samples which were examined. In all, about 50 per cent of the original substance was left in the flask as a residue, the remaining 50 per cent consisting of water and oil, the amount of the latter ranging from 22 to 28 per cent. This difference in the oil content may be due to variations

in the manner of gathering the resin, which may permit the evaporation of an indefinite quantity of water, or may be caused by differences in the composition of the resin taken at different times of the year, or from trees of different ages. The residue, after distillation, is a dark-colored, brittle product which evidently is of little value unless it were to yield useful products as a result of destructive distillation. It readily and completely dissolves in chloroform, partially so in ether (leaving a white sediment), not at all in alcohol, and slowly and completely in boiling turpentine. The heating of this resin was continued and it was subjected to a slow destructive distillation, the temperature being so regulated that the thermometer in the vapor did not run higher than 250°. The oil obtained as a distillate constituted 62 per cent of the original by weight and a very small amount of water was formed. On refractionation this product gave 28 per cent below 250° and 40 per cent between 250° and 300°. Beyond 300° the distillate was of the consistency of rosin oil. The first fraction was of a light-green color and contained a very small proportion boiling below 200°.

The oil first obtained by direct distillation of Balao to 270°, after its separation from water, passed over almost completely between 260° and 264° (760 millimeters). No low boiling oil was present. A sample fractioned twice under reduced pressure showed a boiling point of 151° to 154° at 40 millimeters.

Its optical rotation was 78°.5 (10 centimeters, 30°) and its specific gravity $\left(\frac{30°}{30°}\right)$ = 0.9127. It was colored a slight yellow and had the characteristic odor of Balao. Another purified, specimen obtained from a different sample showed the same boiling point. It had a rotation of +87° (10 centimeters, 30°) and a specific gravity of $\left(\frac{30°}{30°}\right)$ 0.9131.

During the removal of this oil from the resin it was necessary to subject the latter to a high temperature by heating with a free flame. Such a process is not favorable to the isolation of a substance in the pure condition, and, as already stated, the oil can not be removed from the resin with steam; so that an expedient was resorted to which consisted of mixing it with equal parts of another non-volatile oil by which means it was possible to isolate the volatile matter from the resin at a lower temperature. Coconut oil which had been thoroughly treated with steam, so as to remove all traces of volatile matter, was used.

The product so obtained redistilled at 149° to 152° (37 millimeters. It was light yellow in color, showed a rotation of +61.3° (10 centimeters, 30°) and a specific gravity of $\left(\frac{30°}{30°}\right)$ 0.9140.

The range in boiling point of this oil is greater than is usually shown by a single, pure chemical substance, but considering its origin and its point of ebullition, it unquestionably is a sesquiterpene, or a mixture of

this class of bodies. Its deportment toward dehydrating agents and toward metallic sodium shows that it contains no substances of an alcoholic nature; furthermore it readily adds halhydric acids and bromine. Despite many attempts, it was not possible to secure from the oil a solid derivative which could be purified by crystallization. Products similar to this one are found in many of the essential oils of commerce, but besides cadinene, which has already been considered in a previous part of this article, in only one case have investigators been able to isolate, either directly or indirectly, an individual substance from such products. By treating certain fractions obtained from oil of cloves and oil of copaiba with a mixture of acetic and dilute sulphuric acid, Wallach[3] isolated a crystalline substance having the empirical-molecular formula required for a sesquiterpene hydrate, the hypothetical mother substance of which was named caryophyllen. The application of this treatment to the oil from Balao did not give rise to a solid derivative. A number of definite bodies, having the general formula $C_{10}H_{16}$ and known as terpenes, have been shown to exist, but it is very seldom that they can be separated in a pure condition by the ordinary physical methods. The application to sesquiterpenes of the methods by which the individual terpenes have been successfully isolated and identified has not led to fruitful results, and a very interesting field is here offered for a more exhaustive study of this interesting and important class of bodies, with a view primarily of identifying and separating the individual components of the sesquiterpene mixtures.

MALAPAHO: OIL OF PANAO.

Several samples of this product, each accompanied by botanical material, were examined. The species was determined to be *Dipterocarpus vernicifluus* Blanco. The resin does not appear to be so widely used as Balao, probably because it dries much more slowly. The method applied to its extraction from the tree is identical with that used for Balao; it is said that a flow of a gallon per day is sometimes obtained. The fresh resin is a white, viscous, sticky fluid having a characteristic odor which serves to distinguish it from other similar products. It absorbs oxygen from the air and on standing becomes dark brown in color. Even when exposed in a very thin film, it hardens very slowly. On heating the resin to 100° its mobility increases, this behavior being different from that of Balao. It appears completely to dissolve in ether and chloroform with the exception of the separation of water; it is only partially soluble in alcohol and benzol; none of its constituents dissolve in water. All of the different samples were found to consist of water, sesquiterpene oil, and solids. On subjecting the resin to distillation with a free flame its behavior is similar to that of Balao. A fresh sample from Ambos Camarines gave 25 cent of water, 35 per cent of oil, and 40 per cent of solid

[3] *Ann. d. Chemie* (*Liebig*) (1892), 271, 288.

residue. The distillation was discontinued at the point where decomposition became evident. Another sample showed a somewhat different percentage of water and of oil, the difference probably being due to the time and method of collecting.

The sesquiterpene oil obtained from Malapaho redistilled almost completely between 256° and 261° at 760 millimeters, which is a little lower than the boiling point of the oil from Balao. On further purification under reduced pressure a perfectly colorless product was obtained, boiling almost entirely within 3°. Specific gravity, $\left(\frac{30^\circ}{30^\circ}\right) = 0.9165$. Rotation, —54° (10 centimeters, 30°).

The solid product resulting from the distillation of Malapaho is similar to that from Apitong, although lighter in color. When subjected to destructive distillation it yields about 50 per cent of a liquid which partly refractions between 200° to 300°, leaving a residue which has the consistency of rosin oil. As was the case with Balao, it was not possible further to identify any of the constituents of the volatile oil of Malapaho.

GENERAL.

The products considered in this article are to be classed with two well-known commercial substances, namely, the balsams of copaiba and gurjun. The Oil of Supa more nearly resembles these balsams than do the other products, since it contains no water and the residue left after distilling the volatile oil is viscous. The *Dipterocarpus* wood oils are composed of a large percentage of water, and after the distillation of the oil, leave solid residues.

Balsam copaiba is a wood oil obtained in South America from various species of the order *Copaifera*. The descriptions found in the literature regarding this product and its volatile oil vary so much and are so contradictory that it is impossible to obtain anything but a very general idea concerning it and its constituents. In general, the balsam may be said to consist of resinous bodies dissolved in a large proportion of a volatile oil which boils between 250° and 275° and may be removed by direct distillation. No definite substances have ever been identified in it or isolated from it excepting the hypothetical sesquiterpene caryophyllen. A sample of copaiba balsam purchased from a drug house in Manila was a dark-colored, transparent, homogeneous liquid. The volatile oil contained in it was removed by distillation under reduced pressure and constituted 38 per cent of the total. There was no water in the product. The oil redistilled within 11° (126° to 137° at 20 millimeters) and was practically colorless. The nonvolatile portion of the copaiba balsam was viscous.

Gurjun balsam (also known as Indian wood oil) is a product widely used throughout southern Asia. Within comparatively recent years it has been introduced into Europe, and is now a regular article of commerce. It is stated to be derived from different species of the *Dipterocarpus* family. The product has never been the subject of thorough

chemical study and the few superficial examinations which have been made and recorded do not show much agreement, other than that the volatile oil boils within a narrow range of temperature at about 255°. The balsam is used in India for preparing varnishes and in adulterating essential oils. It is often stated to be sufficiently mobile to permit of filtration, to possess a fluorescence, and to coagulate on heating. A sample of this product purchased from a drug firm in Manila was found to contain 74 per cent of volatile oil capable of being removed by direct distillation. Only a trace of water was present and the residue was viscous.

The violatile oil redistilled within 2° (154° to 156° at 48 millimeters) and showed the following constants: Rotation, +53° (10 centimeters, 30°). Specific gravity, $\left(\frac{30^\circ}{30^\circ}\right) = 0.9103$.

This gurjun balsam was completely soluble in all the ordinary solvents excepting ligroin, in which it showed a decided turbidity, differing in this respect from the sample of copaiba balsam and also from Oil of Supa; it is also distinct from each of these products in the composition of its volatile oil, as is indicated by the boiling point. Gurjun, as well as copaiba, is produced by making a deep cut, or cavity, in the trunk of the tree near the base. Into this the resin exudes rapidly, amounting in individual instances to from 50 to 180 liters.[4] After the exudation has diminished, a fire is built in the cavity or on the ground below it, which means greatly increases the flow.

From such a general knowledge of the balsams of copaiba and gurjun as it is possible to obtain, it appears that Oil of Supa is a very similar product and that it should prove valuable for the same uses. Copaiba and gurjan, as well as the oil distilled from them, are used for like purposes in medicine. Considering that these oils are not identical and possibly have nothing in common, except that they belong to the class of sesquiterpenes, it seems very probable that the volatile oil from Supa could be put to the same uses. The Oil of Supa could also be utilized in the ways mentioned, namely, in making varnishes, paints, and transparent paper, and in the adulteration of other oils.

In connection with these products the resin from the *Dipterocarpus* tree, Mayapis (Tagalog), is of interest. This product is considered by Dr. Tavera in his book "Medicinal Plants of the Philippines" to be identical with gurjun balsam. A sample of the resin from Mayapis was obtained from Bataan Province. On examination it proved to be quite similar to the two *Dipterocarpus* wood oils which have already been considered. It contained 15 per cent of water and 25 per cent of sesquiterpene oil, which could be removed by careful distillation without decomposition. The residue was quite hard.

[4] Wiesner: *Die Rohstoffe des Pflanzenreiches.*

The oil redistilled at 17 millimeters, possessed the characteristic odor of the resin, and was very light yellow in color. Boiling point, 132° to 140° (17 millimeters. Specific gravity, $\left(\frac{30°}{30°}\right) = 0.9056$.

The *mayapis* resin was light colored, apparently homogeneous in composition, and so viscous that it could scarcely be poured. When heated to 100° it hardened, and exposure to the air produced the same effect, changing it to a pearly, white solid. It dries much more rapidly than do either Balao or Malapaho. The scientific name of the tree yielding Mayapis resin is *Dipterocarpus anisoptera vidaliana,* and Mr. Merrill states that this species has never been encountered outside of the Philippines. Most of the properties of the sample I worked with—namely, the high per cent of water, behavior on heating, low percentage of oil, consistency, and boiling point of the oil—are different from those generally given for *gurjun balsam.*

Balao and Malapaho are largely used by the natives of the Philippines for purposes I have already mentioned, but their application is due to a lack of better material. As varnishes they dry too slowly ever to be considered by the side of modern products. Balao is superior to the other in this respect and gives a very tough and durable coating. The drying properties of these wood oils might be enhanced by the addition of drying oils were it not for the fact that the admixture is not practicable. If practical uses are ever discovered for sesquiterpene oils, then these resins may be of value as a source of the latter, also the products of destructive distillation which have been previously discussed probably largely consist of sesquiterpenes, and at the same time the higher boiling and more viscous portions produced by this decomposition could be put to uses similar to those of rosin oil.

Sesquiterpenes.—Bodies of this type in small quantities have been known for many years, as they occur in nearly all the essential oils of commerce, but the latter generally are expensive and their constituents have been looked upon as rare substances. It is unfortunate and strange that the sesquiterpenes of copaiba and gurjun have never been the subject of serious study. The latter products, as well as those considered in this article, are very largely composed of sesquiterpenes, so that if a demand were ever created for chemicals of this type there would be an abundant supply. The sesquiterpenes would probably be inferior to terpenes for most of the present uses of the latter because of their being practically nonvolatile. However, this property might allow of their use as solvents in various industrial operations where turpentine is inadmissible. Just as is the case with turpentine, they dry slowly in the air, forming a tough varnish, and they might, to a limited degree, profitably be used with the latter for the thinning of paints and varnishes.

In this connection one other point is to be considered. The sesquiterpenes have never received the thorough investigation which they merit

as a distinct and widely distributed class of substances, and it does not seem unreasonable to expect that, when they have received such study, important practical uses will follow as a result. There will then be a demand for the products yielding them. It is a very remarkable fact that we have so high a percentage (from 35 to 80) of volatile sesquiterpenes in most of these wood oils, while the resins yielding terpene oils very seldom have a content of more than 25 per cent of the latter, and the proportion is generally much less.

I wish to return thanks to Dr. H. N. Whitford, of this Bureau, for his valuable and willing help in securing some of the material used in this work.

ORBITOIDES FROM THE BINANGONAN LIMESTONE.

(WITH SOME NOTES ON EARLY CONNECTIONS BETWEEN FORMOSA, THE PHILIPPINES, AND JAVA.[1])

By W. D. SMITH.

(*From the Division of Mines, Bureau of Science.*)

On looking over some samples of fossiliferous limestone collected by Mr. H. M. Ickis, of this Bureau, from the classic Binangonan locality, some forms which resembled *Orbitoides* were noted. Some time later it was possible for the writer to make a trip to this same locality, on which occasion he collected more material and obtained some data with reference to the field relations of the formations.

On closer study, the forms were seen to be, without an exception, species of *Orbitoides*, and no *Nummulites* were detected. However, Richthofen [2] may have seen *Nummulites* there also. Thin sections were made and studied in connection with the admirable sections of similar forms from Formosa and the Riu Kiu Group,[3] which were sent by Professor Koto, of the Imperial University of Tokyo, to Messrs. Newton and Holland and described by them.

In 1862 the late Baron von Richthofen visited a limestone quarry about 4¾ mile northeast of the pueblo of Binangonan on Laguna de Bay, and, according to his account, collected some *Nummulites*, and ever since that date this formation has remained unquestioned, save by Mr. Becker, and referred to the Eocene. So far as we know, Richthofen never figured or described these forms.

[1] This paper the writer intends to serve as an introduction to a field of investigation which he has been assigned to develop as time and opportunity permit. This field, as interesting and important as it is from a scientific point of view, must be made subordinate to the economic work which the writer and his colleagues of this Bureau are at present engaged in. However, it is hoped that articles bearing on this and related subjects will from time to time appear in the numbers of this JOURNAL.

Many statements herein may have to be modified as future work progresses, so that the present conclusions should be regarded more in the light of a working hypothesis than as a definite and final opinion.

[2] *Zeitschr. d. geol. Gesell.* (1862), 14, 357-360.

[3] R. B. Newton and R. Holland: "On Some Fossils from the Islands of Formosa and Riu Kiu." reprinted from *Jr. Coll. Science Imp. Univ. Tokyo* (1902), 17, art. 6.

This is not the first instance of the finding of *Orbitoides* in the Philippines, for in 1901, Mr. Martin,[4] the recognized authority on the little-known paleontology of these Islands, published a short statement concerning *Orbitoides* which were found by Semper in a marl from Alpaco, Cebu.

The importance of *Orbitoides* in the Philippine and Malayan stratigraphy can not be brought out too strongly, for it is a typical zone fossil—i. e., widely distributed, but restricted in vertical range—and from it we have been able to make some interesting and highly important correlations which will be mentioned in the following pages.

FIELD RELATIONS.

Binangonan is situated on the western side of the western of the two peninsulas which extend southward into Laguna de Bay, due east from Cavite. It is reached by launch from Manila by way of the Pasig River.

The surface rock throughout the country immediately north of Laguna de Bay is volcanic and consists of very recent trachytic and basaltic flows, while farther to the north and south is a vast tuff area, familiar to geologists from the literature of Abella, Von Drasche, Semper, Becker, and others. As one goes northeast along the old trail to the limestone, the ground rises rather gradually, until the backbone of the peninsula is reached, at an elevation of about 350 feet, from which altitude the surface drops away in a series of poorly preserved terraces to a broad, flat-bottomed valley on the east.

That this valley was at one time an arm of the Laguna and also of the sea there seems to be little question, for on the highest bench just below the limestone cliff (fig. 1) two shells belonging to the genus *Crassatellites* (marine) were found.

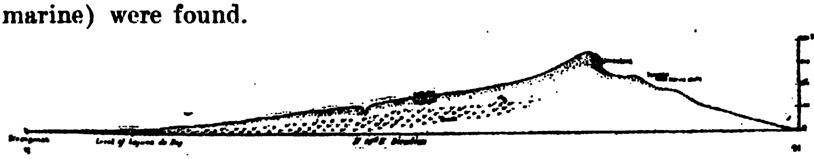

FIG. 1.—Ideal section of part of Binangonan Peninsula.

Almost identical species are living in Philippine waters to-day.

On the western slope of the peninsula also, the evidence of recent uplift is indicated by the deep U-formed stream gorges.

THE VOLCANIC ROCK.

At Binangonan the lava is a dense, bluish-black, clean-cut basalt very much like some phases of the rock from Talim, but a little to the northward in the old city (Manila) quarry, whence the rock was taken for road metal, it becomes lighter colored and more cellular. A section of this has been examined with the petrographic microscope and found to be

[4] K. Martin: "Orbitoides von den Philippinen." *Centralblatt für U. G. P.* (1901), 326, 327.

typical olivine basalt. (Pl. II, fig. 2.) The principal minerals are labradorite, olivine, a green augite, and magnetite. The trachytic texture is very pronounced in the thin section. Zonal structure is very common in the feldspars and an occasional twin in the shape of an X can been seen.

As we travel up the slope to the divide we find the lava becoming more porous and lighter in color, until in the neighborhood of the limestone it is practically a scoria. From the rather limited observations the writer was able to make here, it appears that these flows probably poured out over the country from Talim, leaving a small peak of limestone in part exposed.

THE LIMESTONE.

The limestone, which is the tomb of *Orbitoides,* is exposed in a cliff-like mass, a hundred feet or more in height, and seemingly dipping steeply to the east, though this may prove to be not true bedding, but some secondary structure. In color the rock varies from a light cream to a dirty, bluish-gray. The lighter and denser portions are more fossiliferous. On microscopic examination it was found to contain *Orbitoides,* differing somewhat from the forms described by Messrs. Newton and Holland, and which the writer proposes to call *Orbitoides richthofeni,* some fragments of *Operculina complanata?* Def., and a very imperfect form which is suggestive of *Lithothamnium ramossisimum* Reuss.

DESCRIPTION OF SPECIES.

The genus *Orbitoides* differs in one radical respect from *Nummulites,* namely, in that the chambers of *Orbitoides* are arranged concentrically and not spirally as in the latter form. All the specimens we have found belong to the Lepidocycline group, this terminology referring to the lozenge-shaped chambers along the median plane. It is probable that more than one species is represented, and there is a great difference in the size of some of the specimens.

Orbitoides richthofeni sp. nov.

(Pl. I, fig. 1.)

The type of this is the largest specimen found in this locality, but unfortunately it is not a perfect one. The one we have depicted by Plate I, fig. 1, has lost a portion at each extremity, but if restored would measure in the neighborhood of 36 millimeters in length and 8 millimeters in width at its thickest portion. These tail-like appendages are very characteristic and give to the whole the appearance of the head of a pick.

The initial chamber is not shown, or it is exceedingly small. Instead, along the median plane are developed lozenge-shaped chambers arranged at right angles to the long axis of the form and continued out into the

caudal appendages. The remainder of the chambers are considerably larger and are in certain sections roughly pentangular in outline. Plate I, fig. 2, shows the central portion of the large specimen, much enlarged. In the photomicrograph it is the black band running through the center. Just what the meaning of this is, the writer is unable to determine at the present time, as he has not seen it in a sufficient number of specimens to ascertain whether it is accidental or is some characteristic feature.

Plate I, fig. 2, shows one of the commoner, smaller forms measuring approximately 8 by 4 millimeters. This is unusually circular, but many specimens are almost identical with those figured in Plate 1, fig. 4, of Newton and Holland's paper, and coming from Irometé Island.

We have as yet seen none from the Philippines to correspond to *O. angularis*, figured on Plate I of their paper.

CONCLUSIONS.

As von Richthofen merely mentioned his having discovered *Nummulites* in the Binangonan limestone and never discribed, nor, to our knowledge, figured, any of the species, and as we have not yet found a *Nummulite* from that horizon, we can not find much evidence for calling this formation Eocene.

Furthermore, Orbitoides (*O. verbeeki* Newt. and Holl.), probably the same as our smaller forms (Pl. II, fig. 1), have been found in limestone, in the Riu Kiu Group, which the British paleontologists, Newton and Holland, have placed in the Miocene, and they have been encountered still farther north, in Japan, with *Lithothamnium*. Also Martin[5] has declared the orbitoidal marl of Cebu equivalent to the "Java Gruppe" in which *Vicarya callosa*, the type fossil of the Miocene, was found.

In this connection it is both interesting and due to Becker,[6] who, though he was greatly handicapped in his work at the time of his stay in the Islands by the unsettled state of the country, nevertheless saw enough to make suggestions invaluable to all succeeding workers, to quote him.

> I must confess that the paleontological evidence as to the existence of the Eocene in the Philippines seems to me far from satisfactory. * * * I can see no reason as yet why the Binangonan limestone may not be Oligocene or even Miocene.

Very recently the writer has examined some sections from the Benguet and Lepanto limestones which Mr. Eveland, his colleague, submitted to him, and which lead him to think it quite likely that these beds are the northward extensions of the Binangonan formation. This is not surprising, for we should certainly expect some intermediate occurrences between the Riu Kiu Group and southern Luzon. In certain beds of limestone

[5] *Loc. cit.*

[6] G. F. Becker: "Geology of the Philippine Islands," *21st Ann. Report, U. S. G. S.* (1902), 552.

in the coal field of Batan Island[7] the writer also found *Operculinae*, although as yet no *Orbitoides*.

The fact that Martin's *Orbitoides* came from a marl, while these we are at present describing occur in a limestone, does not in the slightest degree prevent the inclosing beds from being contemporaneous although they may not be strictly homotaxial.

The bearing of these facts upon the paleogeography, and consequently upon the distribution of the flora and fauna of these Islands, would seem to be exceedingly important. If it can be satisfactorily proved, and these facts appear to contribute something to that end, that the islands of this Archipelago are remnants of a former, more extensive, land mass which was connected with Formosa and Japan to the north and Borneo, Java, and the Malay Peninsula to the southwest, and even with Indo-China and India, much that is now problematical with regard to floral and faunal distribution in this region will have been solved.

This highly interesting problem has been attacked by many naturalists, foremost among whom are R. A. Rolfe[8] and A. R. Wallace.[9] These authors have demonstrated the great and almost confusing mixture of Australian, Indian, Chinese, Formosan, and still more northern types of plants and animals, more particularly the latter, with the endemic forms of the Archipelago. These will not be detailed here, but we shall discuss the distribution and origin of some of these forms.

Mr. Wallace gave two views as to the ancient geography of this Archipelago, one of which, expressed in 1876,[10] maintains that the Islands are truly insular and volcanic and that the union with other Malayan Islands was not of such a nature or duration as to permit of any extended migration on the part of animals. Later, in 1902 in the third edition of Island Life,[11] he gives expression to a second view as follows:

> It is evident that the Philippines once formed part of the great Malayan extension of Asia, but that they were separated considerably earlier than Java and have since been greatly isolated and much broken up by volcanic disturbances; their species have for the most part become modified into distinct local forms, representative species often occurring in the different islands of the group. They have received a few Chinese types by the route already indicated, and a few Australian forms owing to their proximity to the Moluccas. Their comparative poverty in genera and species of the mammalia is perhaps due to the fact that they have been subjected to a great amount of submersion in recent times, greatly reducing their area and causing the extinction of a considerable portion of their fauna.

[7] W. D. Smith: "The Coal Deposits of Batan Island," *Bull. Min. Bur.* (1905), No. 5.

[8] R. A. Rolfe: "On the Flora of the Philippine Islands and Its Probable Derivation." *Jour. of the Linnean Society, Botany* (1884), 21, page?

[9] A. R. Wallace: *Island Life.*

[10] A. R. Wallace: *Geographical Distribution of Animals* (1876), 1, 344.

[11] A. R. Wallace: *Island Life*, 3d edition (1902), 389.

Mr. Rolfe, writing in 1884, favored the former of Wallace's views, but states that "geological evidence will probably in future throw much light on this point."

It appears to the writer that Wallace's later view is more nearly in accordance with the facts. He was familiar at that time with the presence of submerged banks connecting the now isolated groups, but he did not have any paleontological evidence.

Rolfe repeatedly speaks of migrations of southern types from the Malayan and Australian regions northward to the Philippines, but from lack of material he was unable to discuss the great infusion of northern types which must have migrated southward from Siberia, and even North America, through Japan, south China, and Formosa, and which are found in the highlands of northern and central Luzon.

The writer, accompanied by Mr. Merrill, botanist of this Bureau, has recently made the ascent of some high mountains in northern Luzon, where many species of plants identical with, or closely related to, those of Formosa, southern China, and Japan were observed, and also some identical with North American forms, which apparently have migrated from that region by way of the Aleutian Islands, Japan, and Formosa to Luzon.

That there have been in the past repeated land connections between Japan and North America, by the closing of Bering Straits, has substantially been proven by the periodic migrations of molluscan faunas between those regions in past geologic periods.[12] Of course, at the inauguration of the glacial period these plants and animals would migrate far to the south and even into the Tropics. It is expected that future paleontological work will corroborate this view by revealing a decided infusion of Japanese forms in the molluscan fauna of the Pliocene and Pleistocene beds.

If there were such a land connection in Miocene times, as we have already indicated, it is probable that it continued nearly to the time of the present flora, previous to which disruption may have taken place through volcanic disturbances, this break occurring early enough, however, to allow sufficient time to elapse during which the flora and fauna of these Islands could take on their present insular aspect.

We should not fail here to refer to work in a somewhat different field—namely, to the investigations on the distribution of the avifauna in these Islands—made during a number of years by Messrs. Worcester and Bourns.[13] Although their work gives evidence of a great break between the Philippine and Bornean groups, we do not believe their

[12] J. P. Smith: "Periodic Migrations Between the Asiatic and the American Coasts of the Pacific Ocean," *Am. Jr. Science* (1904), 17, page?

[13] Dean C. Worcester and Frank S. Bourns: "Contributions to Philippine Ornithology." *Proc. U. S. National Museum* (1898), 20, 549.

conclusions are greatly at variance with our own, for the present distribution of the avifauna might not, and probably did not, go very far back in point of time. Furthermore, birds being far more capable of migration than plants or invertebrate animals, their distribution could not be considered as having as much weight in the evidence as that of the latter. Unfortunately, these investigators did not extend their observations beyond this Archipelago, so that we do not know what their views would have been on this broader problem.

However, it should be stated that the conclusions given above can only be tentative until more is known of the ancient faunas and flora of these Islands and until further study of a comparative nature of the present fauna and flora of China, Japan, Formosa, and the Philippines has been undertaken. It should be stated that Mr. Merrill is now carrying on this work on the flora, and the results of his investigations will be awaited with great interest by all naturalists.

In further support of Wallace's view should be mentioned the occurrence reported, and presumably in the Miocene of Mindanao, of remains of *Elephas* (*Stegodon*) recently identified by Professor Osborn, of the American Museum of Natural History. This *Stegodon* formerly ranged all through southern Asia and is the ancestor of *Elephas indicus*, the living elephant of India.

ILLUSTRATIONS.

(Photomicrographs by Martin.)

PLATE I.

FIG. 1. *Orbitoides richthofeni*, sp. nov.
2. *Orbitoides* sp.?

PLATE II.

FIG. 1. *Orbitoides verbeeki* (?) Newton and Holland.
2. Binangonan basalt.

FIG. 1.

FIG. 2.

PLATE I.

FIG. 1.

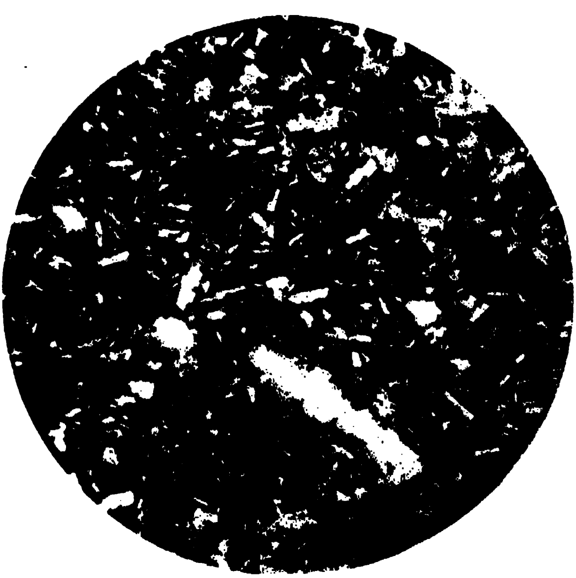

FIG. 2.

PLATE II.

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THE PRINCIPAL INSECTS ATTACKING THE COCONUT PALM (PART II).

By CHARLES S. BANKS.

(*From the Entomological Section of the Biological Laboratory, Bureau of Science.*)

In Part I of this paper, insects which attack the trunk and the undeveloped leaves and flower clusters of the coconut were discussed. All the forms which have been described belong to the Coleoptera, but there are also certain species of Lepidoptera and Coccidæ which attack the coconut to a sufficient extent to warrant their being designated as injurious.[1]

Two forms of Lepidoptera are found upon the leaves of the coconut, one belonging to the Rhopalocera and the other to the Heterocera; the first is the coconut skipper, *Padraona chrysozona* Plötz, of the family Hesperiidæ, and the second, *Thosea cinereamarginata* Banks, of the Limacodidæ. While the nature of the damage done by the caterpillars of these two forms is very similar, the insects differ entirely from each other both in the larval and adult stage. Neither is likely to prove a very serious menace to the life of the tree. Each attacks the leaflets after they are practically full grown. A single caterpillar confines itself to a single leaflet until, with the exception of the midrib, it has entirely devoured it, whereupon it proceeds to another, and so on until the caterpillar has attained full growth. In the case of the coconut skipper, the caterpillar not infrequently eats a space from the blade of the leaflet at a point near its attachment to the main petiole, leaving the distal part untouched. (See Pl. I.)

[1] The bibliography of coconut insects, appended, includes all forms known to attack the tree, either here or in other countries, and is intended to be of further aid to those interested in the subject from an economic standpoint.

THE COCONUT SKIPPER.

LEPIDOPTERA.

HESPERIIDÆ.

Padraona chrysozona Plötz.

During the months of September and October many of the leaflets of small coconut trees of from 6 to 15 feet in height are partially destroyed. Certain of these leaflets have their outer edges sewn together by means of a pure-white silk which is decidedly elastic, so that the leaf may be pulled slightly apart without tearing the fastening. Inside these folds the light, yellowish-green caterpillar, having a chitinous head, somewhat darker than the body and boldly marked with a very regular pattern, is encountered.

Toward the latter part of October the semiactive pupæ are found in these "cradles," partially covered and surrounded by a snow-white flocculent substance, which has a wax-like feel. This substance has very much the appearance of the wax secreted by certain species of Coccidæ and is exuded from the skin pores of the caterpillar toward the end of its larval stage. It serves as a protection for the pupa.

The coconut skipper, like nearly all Hesperiidæ, flies during the very early morning or the late afternoon and early evening hours, and hence it is very difficult to observe its egg-laying habits.

The eggs are found upon the under side of the leaflets of the coconut and but rarely more than one occurs upon a single leaflet. They hatch in from seven to eight days and the young caterpillar, after devouring all of the eggshell except that portion in contact with the leaf surface, at once proceeds to the edge of the leaf and begins to feed. This process consists in cutting out an oblique swath extending toward the midrib, of about the width of the insect's head. Frequently the caterpillar abandons a portion of the leaf, after having fed upon it for a short time, the result being that leaflets are encountered the margins of which are deeply notched, as shown by Plate II, fig. 1. Under normal conditions, and after the caterpillar has cut the leaflet to the midrib, it sews the margins together to form its nest, feeding upon the cut edge, either toward the apex or the base of the leaflet.

The neck of this caterpillar is much constricted, and therefore the head has considerable freedom of motion, but in a state of repose the normal dorso-ventral axis of the head is so inclined that it lies nearly in a plane with the longitudinal axis of the body, thus causing the mouth to be elevated and projected forward to form the extreme anterior point of the insect (Pl. II, fig. 3 A), which, in such larvæ as those of *Attacus atlas* Linn., and *Thosea cinereamarginata* Banks, is formed by the front of the face or the occiput. The caterpillar of the latter has the head deflected beneath the body.

Description and Life History.

Egg (Pl. II, fig. 2 and 2 A.)—Diameter 1.05 millimeters, height 0.85 millimeter; of a rather flat, oblate-spheroidal shape, yellow-glabrous when first laid, with crimson, subcentral ring and central spot covering the micropyle, and developing after two days. The under surface nearly flat, glabrous; upper surface minutely punctured. It adheres strongly to the leaf surface.

The eggs of *Padraona chrysozona* Plötz are always laid singly upon the leaf, thus differing from those of *Erionota thrax* Fabr., which may be found in groups of from 8 to 15.

The period of incubation is from seven to eight days.

Larva (Pl. II, fig. 3).—Length 3.5 millimeters, width of head 1.2 millimeters; upon emerging from the egg. At this stage the larva is of a pale, greenish-yellow, with a black head, the size of which appears somewhat disproportionate to that of the body. A very fine, light-grey, sparse pile covers the body, especially the posterior segment.

The full-grown larva measures 45 millimeters in length and 4.5 millimeters in width, the head being 3.75 millimeters in diameter. It is of a pale, ocher-green, semi-transparent, permitting the viscera, especially the heart and the malpighian and urinary glands, to be seen readily through the skin. The head, which is about one-tenth the length of the entire body, is biscuit shaped or of a very flat, oblate-spheroidal. The surface is strongly and coarsely punctured. The ecdysical sutures are strongly marked by narrow sulci. It is of a glabrous, tawny, flesh-color. A dark-brown line extends from the base of each mandible around the side of the head to the occiput, where it is deflected forward, following the ecdysical suture and being again deflected toward the side of the head, ending in a sharp point, the lines of each side thus forming a Y on the median, dorsal aspect of the head. The ocelli, which lie in the beginning of the dark line posterior to the mandibles, are 6 in number on each side and of a dark-brown. The mouth parts are dark-brown and glabrous and are surrounded by a rather coarse, porrect pile. The anal segment is glabrous and its posterior margin is strongly rounded, with numerous, white, curved setæ projecting from it posteriorly. The legs are light yellowish-buff with many white setæ on their lower surfaces. The abdominal feet, of which there are 10, are strongly pubescent. The spiracles, which are functional on the 1st and 4th to the last body segments, are of a light yellow.

Pupa (Pl. II, fig. 4 and 4 A).—Length 25 millimeters, width 4.5 millimeters. The pupa is of a glabrous, dark-brown, but is frequently so covered with a white, flocculent substance that its true color is not apparent. It is strongly seto-pilose, especially on the anterior dorsal part of the head and thorax and on the abdominal segments. The setæ upon the thorax project anteriorily, those upon the abdomen posteriorly. Very dark-brown rings extend around the apical margins of the 4th, 5th, and 6th segments. The proboscis extends to the apex of the 6th, its apical fourth being force free and traversely rugose.

A very remarkable feature of this pupa is the form assumed by the prothoracic spiracles. They are completely protected by a reniform patch of dense setæ and are located one on each side of the posterior dorsal margin of the pronotum. See Pl. II fig. 4 B.

Imago, male (Pl. III, fig. 1).—Length of body 15-16 millimeters, length of fore-wing, 15.5-17.5 millimeters. Ground color, bright, yellow-ocher, with the following dark-brown markings or suffusions: The veins and a more or less obliterated longitudinal patch along the posterior part of vein VII_1, and VII_2, from the base for one-half its length, in some specimens suffused with yellow-ocher; a similar, somewhat wider patch from the end of the cell to a point its own length from the outer margin. In some specimens this patch is confluent with the basal

one, forming an irregular, oblique band across the disc of the wing from the base to near the apex. Exterior to this wide patch, a yellow-ocher, irregularly rhomboidal spot, divided by the dark-brown vein V_2. A broad, marginal, dark-brown band from the costa to the posterior angle of wing, its inner margin scalloped and its area crossed by ends of veins which show yellow-ocher; a dark-brown suffusion at the base of and posterior to vein IX. Cilia, black, suffused with yellow-ocher toward the apex and yellow-ocher toward the posterior angle. The inner margin clothed with long yellow-ochraceous hairs. The hind wings with ground color dark violet-brown at the base, with iridescent, subcostal scales. Margin, dark-brown; cilia, yellow-ocher. A longitudinal, yellow-ochraceous patch in the cell, from which spring long hairs of the same color. A broad, yellow-ochraceous, irregular patch extends across the wing from the outer third of vein III to the middle of vein IX, which it follows to the base and margin of the wing respectively. Posterior to the cell patch is another one of long, yellow-ochraceous hairs extending to the oblique band. A similar patch extends along the inner submarginal area its own width from the inner margin of the wing.

The under surfaces of wings yellow-ocher; veins, marginal hair line, base and inner margin to vein IX and submarginal series of suffused spots on fore wing, dark-brown. On the hind wing the superior, oblique, yellow-ochraceous patch has its margin indicated below by series of faint brown spots or irrorations. Antennæ brown, with dark yellow-ochraceous tips and lighter scales beneath. Palpi, sternum, venter, legs and apical margins of abdominal segments, yellow-ocher. Apex of abdomen dark violet-brown with yellow-ocher irrorations. Removal of the head in both sexes reveals a patch of very broad, pearl-colored scales dorsally at the neck. Under normal conditions these scales are completely hidden by the hairs upon the head and thorax.

Female (Pl. III, fig. 2).—Length of body 17.5–20 millimeters, length of wing 18.5–21 millimeters. Ground color, dark violet-brown, especially on the veins of the wings; iridescent in certain lights.

The bases of the fore and hind wings, the head, thorax, the abdomen dorsally and the entire lower surface suffused with greenish-buff. Fore-wings above, with an irregularly, sub-rhomboidal spot in the end of the cell, 2 oblique, parallel lines between this and the costa, 3 parallel patches beyond them, near the costa an oblique, interrupted band, from the outer third vein of V_3 to the middle of inner margin, buff, irrorated with brownish scales. Beyond the oblique band an irregular patch between veins III_5 and V_2 interrupted by vein V_1. Inner margin with greenish-buff hairs for two-thirds its length. Cilia brown and greenish-buff.

Hind-wings as described above, with an ill-defined, interrupted, oblique, buff band from the middle of vein V to the middle of vein IX along which it extends as in the male. Cilia, buff. Inner submarginal area with long, greenish-buff hairs.

Beneath; wings brown, entirely suffused with greenish buff; the markings, which are buff above, are very pale-buff below and both wings have distinct, dark-brown veins and marginal lines.

Antennæ dark-brown above, and buff beneath to the base of club; bases of antennæ, black. Palpi, greenish-buff; apical segment, dark-ocher. Thorax and legs covered with dark, greenish-buff scales and hairs; tarsi, with reddish-brown spines beneath. Abdomen with 5 distinct, transverse, black lines and 3 indistinct apical orange ones dorsally. Anal region with orange scales.

Semper[2] says of this species: "This beautiful species is very easily recognized by the 3 yellow rings of the abdomen. It belongs next to *augiades* Felder and *palmarum* Moore, in which the males also lack the sexual sign [discal patch], upon the forewings, which appears in the female of *augias* L., and *bambusæ* Moore.

[2] Semper: *Reisen auf den Philippinen* (1892), 2 No. 5, 301 et seq.

Upon this ground, I believe, Staudinger's opinion that *bambusæ* is a variety of *augiades* can never be right according to my way of thinking."

"The female of *chrysozona* varies greatly in the width of the dark markings; I have some which are as dark upon the upper side of the forewings as Moore's *palmarum*, others again have, with exception of the border, only fine, black stripes along the veins and a faint, dark shadow on the end of the cell. The ground color is darker than in *palmarum*, and the same as in *augiades*. The female on the upper side is exactly the same as the drawing of *palmarum*, but differs on the underside in that the light bands are nearly as clearly marked as they are above. The ground color is greenish-gray-brown."

This species is also found upon the betel palm (*Areca catechu* L.); in fact, Semper indicates in his note concerning this insect that the larva is only encountered upon that tree. My observations disprove this statement; indeed, it rarely is seen on any palm other than the coconut.

Preventives and remedies.—This insect is never found in sufficient numbers to justify the fear that it may become a serious menace to coconut culture, but as its feeding upon the leaves of small trees may have a tendency to debilitate them, its larvæ should be destroyed whenever they are encountered.

Parasites.—This insect is probably, to a great extent, held in check by two small Hymenopterous parasites, *Chalcis obscurata* Walk., and an unidentified Braconid, both of which attack the larva, laying their eggs within its body, their young feeding upon its fats and body fluids. The larvæ of the former parasite, of which there may be as many as 10, pupate within the pupa of the coconut skipper, which they kill, emerging therefrom in from five to six days thereafter (Pl. IV, fig. 1); those of the Braconid leave their host when they are full grown and, like all true Braconidæ, they spin pure-white cocoons in the vicinity of the now dead and shriveled caterpillar. After spinning their cocoons the insects emerge in about 4 days. (Pl. IV, fig. 2.)

DESCRIPTION OF PARASITE.

Walker's description of the Chalcid is as follows:

Chalcis obscurata Walk., *Proc. Ent. Soc. Lond.* (1874) 399.

Male.—Body, antennæ and legs black, with the usual structure. Body convex. Head and thorax scabrous, dull. Antennæ stout, nearly filiform. Prothorax about four times as broad as long. Sutures of the parapsides, distinct. Abdomen smooth, shining, subsessile, with cinereous tomentum toward the tip. Femora yellow at the tips, hind femora minutely denticulated beneath. Tibiæ yellow, striped beneath with black; hind tibiæ black at the base. Tarsi yellow, tips black. Wings cinereous; squamulæ yellow; veins black; ulna about half as long as the humerus.

Hab.: Hiogo (George Lewis), Philippines (Banks).

This is the first record of this species of the Chalcididæ as from the Philippines.

THE COCONUT SLUG-CATERPILLAR.

LEPIDOPTERA.

LIMACODIDÆ.

Thosea cinereamarginata Banks.

Thosea cinereamarginata Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 229.

The slug-caterpillar is easily distinguished from other Lepidopterous larvæ by the form of its body and its mode of locomotion, which is more like that of slugs or snails than of insect larvæ. Several species are known in the Philippines, many of them feeding upon palms. In Manila, this caterpillar is quite common and is usually found feeding either upon the upper or the lower surface of the leaves of the coconut. It presents a rather forbidding aspect, due to its being well armed with a double series of spinous tubercles placed upon either side, but, as a matter of fact, unlike most Limacodidæ, it possesses no poisonous properties. I have handled the larvæ freely without experiencing any discomfort.

The damage which this insect does to the coconut leaves is about equal to that of *Padraona chrysozona* Plötz.

Egg.—Diameter 1.5 millimeters, height 0.95 millimeters; of a flat, oblate-spheroidal shape and with minute reticulations upon the surface, pale-ochraceous. The larva escapes through a slit which divides the shell across its face, and the latter is not eaten as in the case of *Padraona chysozona* Plötz. The period of incubation is from 5-7 days.

Larva (Pl. V, fig. 1).—When full grown, length, 23.75 millimeters, width, 14.25 millimeters including the tubercles. It is pale-green above and pale greenish-yellow at the sides, being almost pure-white beneath, and with the following markings: a median, light-purple or heliotrope band with symmetrical scalloped margin, the scallops expanding upon the respective segments. The margin of this band is darker purple and shades into the green of the dorsum. The band is developed into more or less of a patch upon the fourth and seventh segments, where the colors are darker. External to this band, on either side, is a series of 9 horizontally-projecting, spiniferous tubercles, those upon the second, fourth, sixth, eighth, and eleventh being twice or slightly more than twice as long as the remaining ones. Below these, ventrally on either side, is a series of 8 light-purple spots, one on each segment from the third posteriorly, and below these another series of 8 spots beginning upon the 4th segment. At the latero-ventral angle, a series of 11 horizontally projecting spiniferous tubercles, one of which projects anteriorly and another posteriorly on either side. The spines of these tubercles interlace and are yellowish-green at their bases and purple or black at their tips. Many of the spines have a white hair at their tips, and the shorter ones at the bases of all tubercles have somewhat inflated tips. The head is yellow-green and when the caterpillar is not feeding, is retracted within the 1st thoracic segment. The length of the larval stage varies from 21–25 days.

Pupa (Pl. V., figs. 2 and 2 A.)—Length 8.5 millimeters, width 5.5 millimeters. The color is a light yellow, with ochraceous bands on the posterior margins of the dorsal, abdominal segments; the wing pads are livid flesh-color and the eyes are dark-gray.

Cocoon.—Length, 8.75 millimeters; dark-brown, cocciform or oblate ovoid, composed of finely comminuted leaf fiber held together with silk. The interior is white and silk lined. The pupal stage lasts about 22 days. Time of flight, the month of January.

Adult.—A description of the adult male and female of this species occurs in the *Phil. Journ. Sci.* (1906) 1, No. 3 p. 220. It is quite closely related to *Thosea minima* Semper, from which, according to Semper's description, it differs chiefly in having the prominent antemedial oblique sinuate band extending from near the cell spot to the middle of the inner margin.

Preventives and remedies.—The same methods of treatment apply to this species as to the Coconut skipper, though, as in the case of the other, there is no possibility of its ever becoming a serious pest.

SCALE INSECTS.

With exception of a few species from which useful or commercial products are obtained, such as the cochineal insect, *Llaveia cacti* Linn., and the lac insect, *Tachardia lacca* Kerr, practically all known species of scale insects are detrimental to man's agricultural interests. In some parts of the world they do more damage to crops and trees than is due to the effects of all the other insects of the region. While, in the Philippines, this is not so strikingly true in the case of the coconut palm, still the damage done to this tree by species of the family Coccidæ is very considerable. It is rare to find a coconut which does not, by its yellow or brown leaves, indicate the ravages of these pests. Scale insects differ so greatly from ordinary insects that they may easily escape detection. As a rule, the commoner forms appear merely as rusty-brown or yellowish patches upon the surfaces of the leaves, or on the bark of the stems or trunk of the plant.

Characters.—In all species the body of the adult female is either covered with a scale formed of a waxy secretion in which the exuviæ of the earlier stages are compacted or else the body of the insect itself assumes a form which suggests a scale or tubercle upon the host plant. The males of all species are winged, but on account of their very minute size and pale colors escape notice unless they are bred upon the food plant under glass, in which case they may be captured upon emerging as adults. The newly hatched young of both sexes are, of course, much smaller than the adults of either sex and it is almost impossible to see them with the naked eye.

Upon hatching, the young, coming from beneath the parent scale, scatter upon the leaf surface in quest of a favorable place to settle. Shortly after their first meal, which is obtained by inserting their probosces into the succulent part of the leaf or twig, the insects shed their skins, but during the period of feeding there will have exuded from certain body pores a pale, wax-like secretion which, adhering to the first exuviæ, after a brief period assumes the form of a scale-like covering.

In the first molt the females usually shed their legs and sometimes their antennæ, so that a female nymph or adult appears as a mere sack, attached to the plant by the proboscis. The female, in those species having a distinct scale, remains under this covering throughout life, while the male, after a succession of molts, comes forth with legs and wings well developed.

In view of the great difference in appearance between the male and the female, it is necessary to consider their respective characteristics separately for purposes of classification, it being impossible to identify two given specimens of different sex as belonging to the same species unless they are found in close association or are bred from a given lot. In view of the relative scarcity of males in most genera of Coccidæ, the characters found in the female form the chief basis upon which their determination is made.

In the Philippines, so far as is known, seven species of Coccidæ are found upon the coconut. Of these, *Aspidiotus destructor* Sign. is by far the most abundant and destructive; next in abundance is *Chrysomphalus propsimus* Banks, a species which has usually been encountered in great numbers on all trees examined both in Manila and in the provinces. The order of abundance of the remaining species is that of the following notes:

THE TRANSPARENT SCALE.

Aspidiotus destructor Sign.

This extremely prolific scale is found on the coconut palm in all localities in the Archipelago where investigations have been conducted. It is extremely injurious to the trees, causing their leaves to assume a characteristic yellow color, which is easily noted from a distance. Where it is encountered, the under surfaces of the leaflets are covered with thousands of small, rough, circular patches, which are almost transparent and so thin that the insect and her eggs can be seen beneath. When the leaflet is pulled longitudinally or when it wilts, the scales become striated owing to the tension on the edges which are attached to the leaflet. Plate VI, fig. 1, shows adults and young scales upon a leaflet. It will be noted that the latter have fixed themselves to the longitudinal veins and therefore are arranged in very regular rows. Fig. 1 A shows young insects which have emerged from beneath the scale of the parent; female scales from which the occupants have been eaten by a tiny predaceous beetle of the family Coccinellidæ, are also present. Fig. 2 shows a coconut leaflet attacked by a form of disease which causes spots, very similar to those resulting from the attacks of *Aspidiotus destructor* Sign., to appear on the upper surface. In case of doubt as to the origin of the spots, certainty is reached by examining the underside of the leaflet, where, if it is attacked by scale insects, the latter will be found just at the point of discoloration of the leaflet.

DESCRIPTION.

Egg (Pl. VII, fig. 4).—Length 0.2 millimeter, width 0.1 millimeter, regularly, bluntly oval, one side more convex, very pale lemon yellow, smooth, laid in two or three more or less regular concentric rows around the parent within the scale. This regular distribution necessitates a nearly complete rotation of the female around the point of insertion of the proboscis. This is accomplished by an undulating motion of the body and may be observed by placing the live insect upon a piece of glass, under the microscope.

Larva (Pl. VII, fig. 8).—Immediately after hatching the length is 0.25 millimeter, the width 0.15 millimeter. Pale yellow, slightly lighter in color than egg. Eyes dark red; antennæ 5 jointed, slightly setose, last joint 3 times as long as the first 4; transversely, microscopically striate, biapical as shown by Plate VII, fig. 5, with a single seta from each apex; mouth two-fifths of distance from frontal to anal margin. Anal margin dentate, giving indication in both sexes of existence of pygidial lobes. These disappear in the male upon the second molt. Legs moderately long, femora somewhat stout. Tarsi single jointed with 2 knobbed spines on the dorsal margin. Proboscis about as long as the body. Four minute hairs project from the frontal and 2 from the anal margin of body, the anal being 4 times the length of the longest frontal.

Male puparium.—Oblong-oval, pale, translucent, larval exuviæ at center slightly darker, yellowish. Plate VII, fig. 3, shows the male puparium.

Female puparium (Pl. VII, fig. 2).—Differs from male in being more nearly circular. Color as in male. Larval exuviæ at or slightly removed from the center. Darker than the scale itself, yellowish.

Signoret's description[a] is very meager. It is as follows:

"The scale is round, of a transparent white, with the exuviæ at the center, and of a yellowish transparent white.

The female is yellow, round; the extremity with six lobes, of which the two median are shortest; the pygidium with four groups of wax glands of eight to ten orifices in each [group] agglomeration."

He says further concerning the insect: "This species appears to cause great damage to coconut groves in the Island of Réunion, where they are menaced with complete destruction. The scale is also found on palms and dates. We have found it likewise on *Goyavius psidium* [*Psidium guayava*] which we received in the same package."

It will be seen that Signoret's description is not sufficiently detailed to differentiate this scale from other very similar ones, as he makes no mention of the squames, their number, and arrangement, which, for example, is a very important point in distinguishing *Aspidiotus destructor* Sign., from *A. lataniæ* Sign. The following description has been prepared from fresh material:

Adult female (Pl. VII, fig. 2).—Length 0.80–0.90 millimeter, width 0.65–0.75 millimeter, bright pale-yellow, broadly oval, nearly circular, narrowed posteriorly, with slight emargination at the base of the pygidium, which is only slightly paler; posterior margin, whitish creamy, due to waxy secretions; two submedian white spots on each side of the genital aperture show the position of the circumgenital glands, the posterior of which have 4 to 5 apertures, the anterior 7 to 9. Anterior margin of the body regularly rounded, abdominal segmentation laterally distinct. Antennæ, small, oval knobs with inwardly curving bristles at the apex, situated one-third of the distance from the frontal margin to the rostrum. Between the

[a] *Ann. Soc. Ent. de France* (1869), (4) **9**, 120; Plate XII, figs. 8 and 8a. Translation.

antennæ on the ventral surface are from 5 to 10 minute spinous tubercles. Parastigmatic glands not present. Stigmata subcylindrical. Pygidium (Pl. VII, fig. 6) with 6 lobes, median pair shorter than the next, weakly tricuspid, light-brown, next pair bicuspid, slightly paler, exterior pair of the same color, bicuspid, of the same length as the median. Chitinous portions of all lobes run anteriorly for some distance into the pygidial area, the surface of which is closely, longitudinally striate. Squames as follows for each side: 2, apically fimbriated, between the median lobes, similar ones, but more slender, between 1st and 2d lobes, 3 stouter between 2d and 3d lobes, with fimbriation somewhat externally laterad of apex; a series of 9 broad, laterally fimbriated ones beyond the 3d lobe and extending one-third the distance from the latter to the base of pygidial margin. These squames decrease in length and increase in width from the third lobe and the number and length of their fimbriations decrease so that the last one bears but 1 prominent spine, the remainder being reduced to sharp serrations of its latero-apical margin. Setæ placed as follows: One pair at the external base of each median lobe nearly twice the length of latter, 1 pair between the second lobe and its 1st external squame, 1 pair ventrad to external lobe, 1 very short, ventrad to the 4th of the exterior 9 squames, 1 ventrad to the last squame, a small setose tubercle near the ventral margin, two-thirds of the distance from the last squame to the base of the pygidium. Four groups of circumgenital glands, the posterior pair with 4 to 6 orifices, the anterior with 7 to 12. Tubular spinnerets filiform; their heads chitinous; tube obconical, chitinous; their tongues one-third the diameter of their heads and equal to them in length. Numerous trumpet-shaped or subcylindrical ducts toward the apex and having orifices on the margin, as do tubular spinnerets. Anal opening about halfway from genital orifice to posterior apex. For details of structure see Plate VII, fig. 6.

Adult male (Pl. VII, fig. 1).—Pale-yellow, with darker, pinkish-yellow, transverse apodema. Head about one-tenth the length of entire body, including genital sheath. Ocelli very dark red; upper pair slightly extra-marginal, lower pair posterior and contiguous on median line, their diameter one-fourth greater than upper ocelli. True eyes posterior to and their own diameter distant from the upper ocelli, submarginal. Antennæ composed of 10 joints, of which the first 2 are subglobular, 3 to 5 subequal in length and about 3 times the length of second, all segments sparsely setose. Tenth segment attenuated, terminating in a clubbed hair, surrounded by 3 other curved hairs of equal length. This is shown by Plate VII, fig. 7. Legs moderately long, posterior femora slightly stouter than the others. The single jointed tarsi, two-thirds the length of tibiæ, slender, subconical and moderately covered with spinous hairs. Tibiæ subequal to femora, with a very few hairs each. Claws or ungues one-fourth the length of tarsi, their digitules two-thirds their length. Tarsal digitule as long as ungues. Genital sheath long and tapering to a sharp point; two-sevenths of the length of the rest of the body. Wings iridescent, hyaline, obovate; veins of about equal length and subparallel to the respective margins. Haltere has the first joint swollen to about one-third its length, just before the apex. Second joint of length equal to the first; spinous and hooked at extremity. Length of wing 0.63 millimeter. This insect so nearly resembles *Aspidiotus lataniæ* Sign. that it is very difficult to separate the two species. The chief points of difference in the female are the number of squames external to the outer lobe, the number of orifices in the circumgenital glands, and the relative length of the median and second lobes of the pygidium, the median in *A. destructor* Sign., being shorter than the 2d pair, while in *A. lataniæ* Sign., they are of equal length and more markedly tricuspid.

Aspidiotus destructor Sign., is by far the most pernicious of the scales which attack the coconut in the Philippines. It most frequently occurs on young trees having from one to five years' growth, in many cases completely covering the under surfaces of all the leaves, giving them a characteristic yellow tinge. It is certain that it is attacked by a Hymenopterous parasite, as female puparia have been found showing the exit holes of the adult parasite, but as yet the latter itself has not been discovered. A small Coccinellid beetle, *Scymnus* sp., is a voracious feeder upon the transparent scale, the adults as well as the larvæ of this species frequently being encountered in considerable numbers upon coconut leaves which are covered with the scales. A description of this insect follows:

Scymnus sp.

Larva.—Length 1.75 millimeters, width 1.01 millimeters, exclusive of the pure-white waxen tufts which project from the front, sides, and posterior margins of the body as shown by Plate VIII, fig. 2. The larva of this beetle when once known can easily be distinguished from all others which might be found among scale insects in this region. It is extremely active, running from place to place and greedily gnawing open the delicate scale in order to obtain the insect which lies beneath. The body is pale yellow with a greyish tinge.

Pupa.—Length 1.25 millimeters, of a light ocher-yellow. This insect pupates within the larval skin, as do many species of the family. In this case the skin splits along the median dorsal line, exposing the pupa.

Adult (Pl. VIII, fig. 1).—Length 1.35 millimeters, width 1 millimeter, of a dark brown, almost black, with a light-brown discal spot on each elytron. In some specimens this spot is sharply, in others ill, defined. The entire body covered with a fine, white, pubescence. Palpi, apices of femora, tibiæ and tarsi, brownish ocher. (See Pl. VIII, figs. 3 and 4 for antenna and palpi.)

Habits. This beetle, the adult as well as the larva, feeds on many species of Coccidæ but has been found in greatest abundance in colonies of *Aspidiotus destructor* Sign.

Chrysomphalus propsimus Banks.

Chrysomphalus propsimus Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 230. (*Pls. I and II.*)

This scale bears a general resemblance to *C. aonidum* Linn., but its color and size, together with its apparent predilection for the coconut palm, upon which it is always found, make its identification as a distinct species a matter of some doubt. The scales crowd themselves upon both surfaces of the leaves of neglected or deformed trees and frequently as many as 4 or 5 are found overlapping each other. In Manila they breed with great rapidity and soon cover the leaflets and even the petioles. The same species has been encountered in great numbers upon the betel palm (*Areca catechu* L.) at San Miguel de Mayumo, Province of Bulacan. It may be distinguished from other scale insects which might be found upon the coconut by the decided, shining red-orange color of the pellicles. The male scales are infrequently met with in comparison with those of the female. (See Pl. X.)

Parlatoria greeni Banks.

Parlatoria greenii Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 231. (*Pl. III, figs. 1 to 6.*)

This delicate, though prolific, scale is frequently seen in Manila upon young, badly cared for coconut trees. It is found upon the upper surfaces of the leaves and because of its flat shape and gray color is difficult to detect. While not as serious a menace as either of the foregoing species, it merits attention because of the possibility of its great increase if it is left unchecked.

Chionaspis candida Banks.

Chionaspis candida Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 232. (*Pl. IV, figs. 1 to 5.*)

Frequently coconut trees are found the partially opened leaflets of which are covered with small, pure-white spots, due to the scales of another species of insect differing totally as to color and form from the foregoing. This scale multiplies rapidly upon either surface of the leaf, usually in the protected parts. As a rule, the female puparium occurs near to a group of male puparia or else with a group of the young scales in their first or second molt (Pl. IX). This insect is not as nomadic as *Aspidiotus destructor* Sign., therefore its opportunity for debilitating the tree is not as great and the danger from it is not to be feared in the same degree as from *A. destructor* Sign.

Lepidosaphes mcgregori Banks.

Lepidosaphes mcgregori Banks, *Phil. Jour. Sci.* (1906), 1, No. 3, p. 233. (*Pls. V and VI.*)

This scale is comparatively rare. It occurs upon both sides of the leaves of the coconut, especially on old ones, but seems to prefer that part of the upper surface that is near to the midrib. It is always encountered singly and the puparia are seldom distorted as in the case with *Chionaspis candida* Banks. Although rare, it may at any time and under favorable conditions propagate to the extent of being injurious. The most noteworthy features which distinguish it are the pair of white, waxy, horn-like projections on the front of the first pellicle, the light color of the female puparium and the regularity of its transverse striæ.

Lepidosaphes unicolor Banks.

Lepidosaphes unicolor Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 234. (*Pl. VII, figs. 1 to 7.*)

This species is of nearly the same shape and size as the preceding, being only distinguished externally by the narrower, interior margin, the color of the puparium and the absence of the waxy horns in old specimens. It is less frequently met with than *L. mcgregori* Banks and therefore less likely to prove a menace.

Paralecanium cocophyllae Banks.

Paralecanium cocophyllae Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 235. (*Pls. VIII to XI.*)

This insect differs from any of the foregoing in that, if a female, it does not lie beneath a puparium but is itself its own scale. It has easily been found upon nearly every coconut examined in Manila, and is readily distinguished from other species by its unusual size (being 5 to 6 millimeters long and nearly as broad), and by the 2 small patches of orange-yellow on the posterior region. It always occurs upon the inferior surface of the leaf. The male puparia are much scarcer than those of the female.

A peculiar characteristic of the male insect is that it comes from beneath its scale to shed the pupal exuviæ, returning after it has completed its transformation. The length of time after the final molt and before it seeks the female, during which the adult male remains beneath the puparium is not known.

PREVENTIVES AND REMEDIES.

In all the work upon scale insects affecting the coconut, it has uniformly been observed that those trees which are ill cared for or which have become deformed by the attacks of beetles are the ones most infested by scales. The malformed or pathologically imbricated leaves, in their interstices, offer ideal places for the breeding of scale insects. This fact would point to the necessity of the removal and destruction of such portions at once.

Because scale insects can only migrate as wingless larvæ, it would seem that their arrest would not be difficult, and yet, when we consider that every wind blows these larvæ from leaf to leaf and from tree to tree, we can easily see that this fact, as well as the extreme fecundity of the insect, renders no tree entirely safe from their attacks. However, those trees which are the healthiest and best cared for are the ones which will best withstand these pests.

Spraying with lime-sulphur or kerosene emulsion washes might serve, if properly applied, for the preservation of young coconut trees, but these remedies would entirely be out of the question for full-grown ones. The necessity is apparent for clean, systematic and regular cultural methods for the protection of this valuable tree from scale, as well as from all other insect pests.

INSECTS AFFECTING COPRA.

In connection with work which has been carried on in this Bureau on coconuts, copra, and coconut oil, it has been noted that certain lots of commercial copra, when received from the bodegas, were badly infested by larvæ, pupæ, and adults of *Silvanus surinamensis* Linn., and *Necrobia rufipes* De Geer. Both of these insects are cosmopolitan and as they

feed upon stored products of a character similar to copra it is only necessary to mention them in this connection.

In the case of these, and of most other injurious insect forms, preventive measures are always most advisable and if care is taken in packing and shipping the copra, receptacles into which the insects can not find entrance being employed, their ravages will be prevented. It is difficult to exterminate them if once they secure a lodgment in a mass of copra. Carbon bisulphide, which might be used successfully against similar insects in grain, would probably prove detrimental to copra owing to its power of dissolving oil.

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ERRATA.

In Part I of this paper:

Pages 161, line 1, and 165, line 14, for Bonn̄gga read Bon̄ga.

Page 166, Table of Illustrations, for Plate I, fig. 1, adults of *R. ferrugineus* Fabr., read Plate I, fig. 1, egg of *Oryctes rhinoceros* L., magnified portion shown at 1*b*.

Plate VIII, fig. 3 should be reversed.

ILLUSTRATIONS.

[All figures are more or less magnified; exact size is indicated in descriptions.]

PLATE I.

Coconut leaf showing work of *Padraona chrysozona* Plötz. Note that on some of the leaflets the apical portion of the blade has been eaten, while in others the basal portion is represented by the midrib alone.

PLATE II. Drawn by W. Schultze.

FIG. 1. Coconut leaflet showing abandoned notches made by young larvæ of *Padraona chrysozona* Plötz.
2. Egg on margin of leaf.
2 A. Profile of egg.
3. Full-grown larva.
3 A. Profile of head.
4. Pupa.
4 A. Lateral view of pupa.
4 B. First thoracic spiracle or stigma.

PLATE III. Drawn by W. Schultze.

FIG. 1. *Padraona chrysozona* Plötz, male.
2. Female.

PLATE IV.

FIG. 1. *Chalcis obscurata* Walk., adult.
2. Coconut leaflet with cocoons of Braconid parasitic on *P. chyrsozona* Plötz together with shriveled caterpillar skin of latter.

PLATE V.

FIG. 1. *Thosea cinereamarginata* Banks, full-grown larva.
2. Pupa.
2 A. Lateral view of pupa.

PLATE VI.

FIGS. 1, 1 A. Coconut leaflet with adult females and young of *Aspidiotus destructor* Sign. Note arrangement of young along veins.
2. Coconut leaflet attacked by disease causing spots similar to those produced by *A. destructor* Sign.

PLATE VII.

FIG. 1. *Aspidiotus destructor* Sign., adult male.
2. Female puparium, showing adult and eggs.
3. Male puparium.
4. Egg.
5. Antenna of larva, distal segment.
6. Pygidium of female.
7. Antenna of adult male, distal segment.
8. Young larva.

PLATE VIII. Drawn by T. Espinosa.

FIG. 1. *Scymnus* sp., adult.
2. Larva.
3. Antenna of adult.
4. Maxillary and labial palpi and labium.

PLATE IX.

Coconut leaflet showing male and female puparia of *Chionaspis candida* Banks. Note group of male scales around female at lower right. A few male and female scales of *Chrysomphalus propsimus* Banks occur also.

PLATE X.

Chrysomphalus propsimus Banks. Male and female puparia on leaflet of coconut.

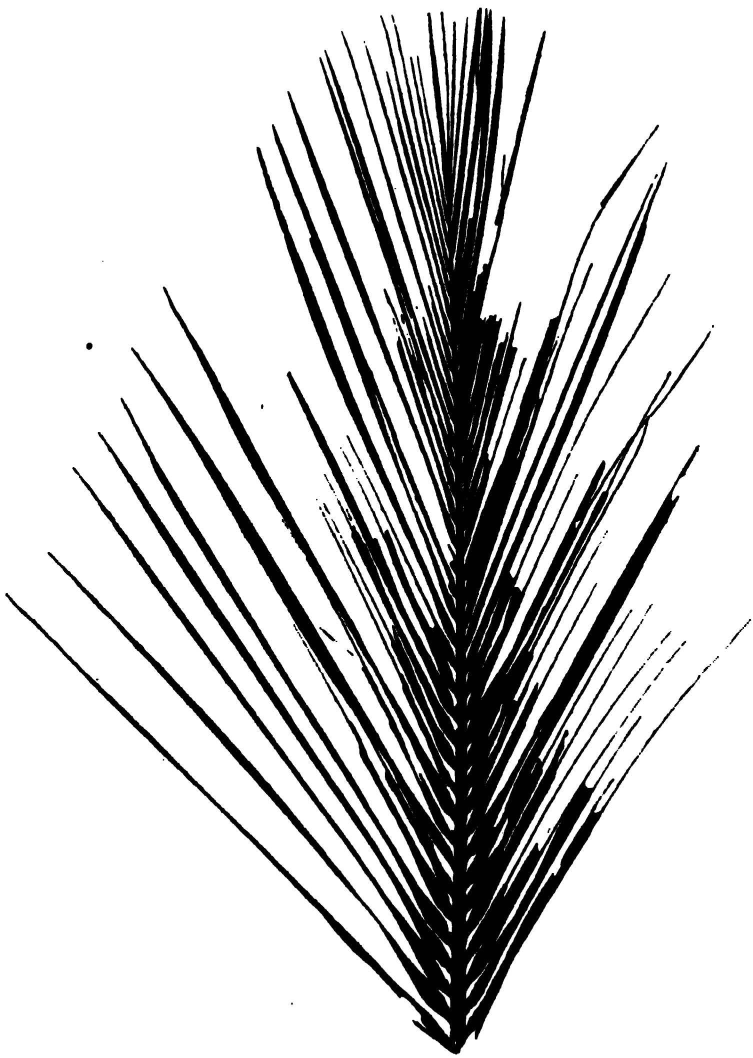


PLATE I.

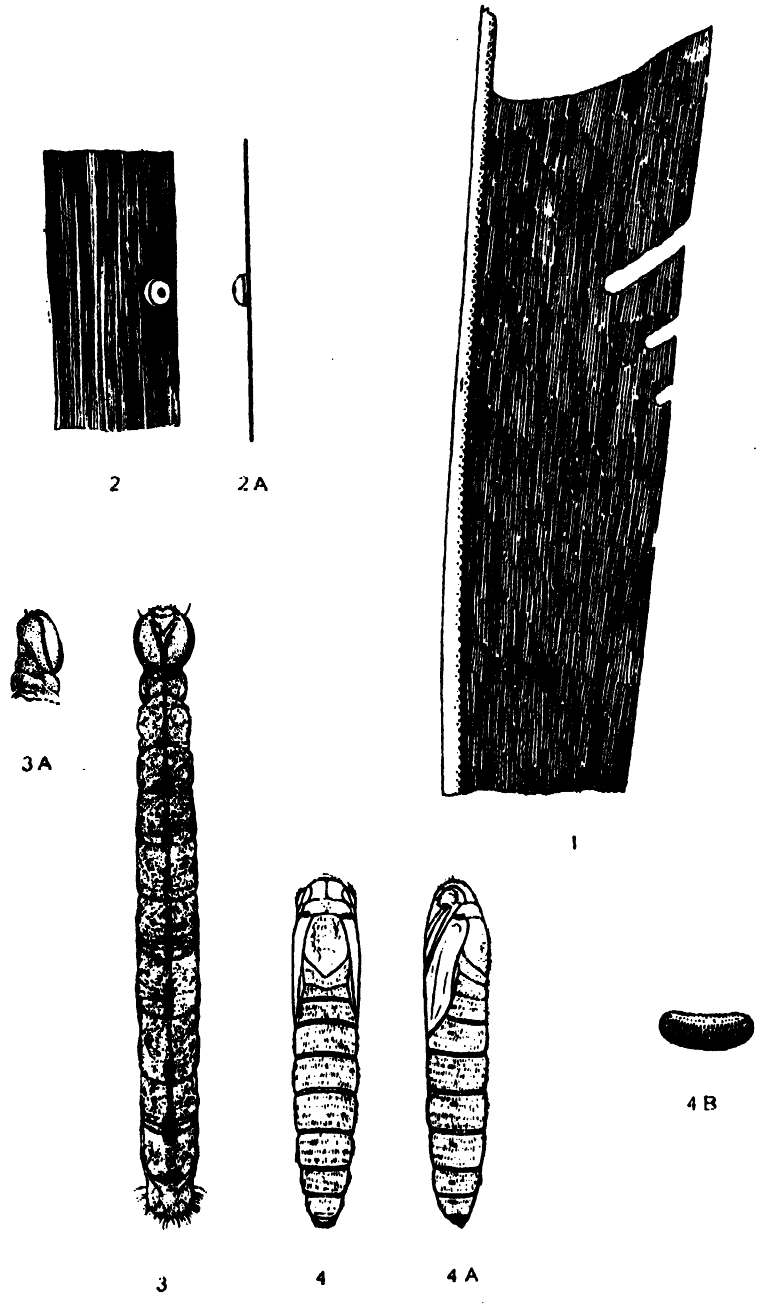
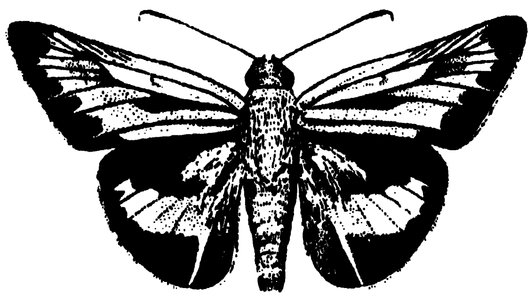


PLATE II.



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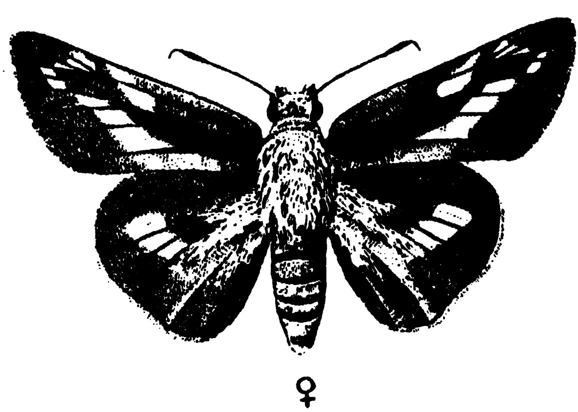

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PLATE III.

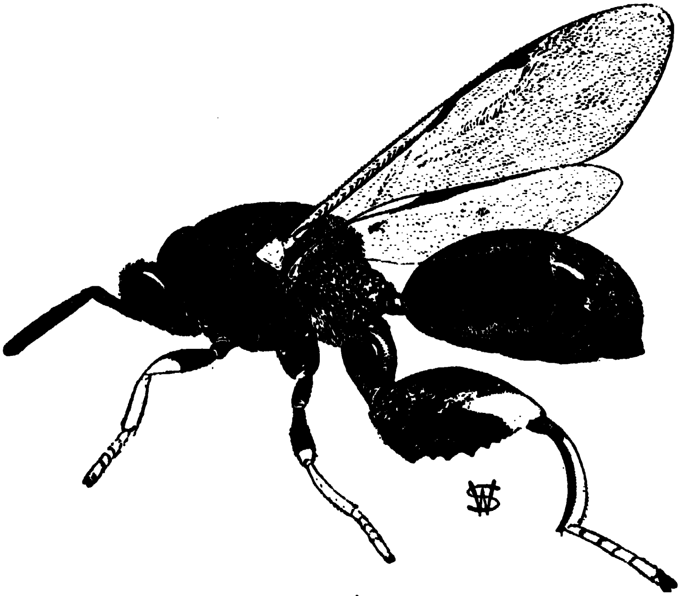

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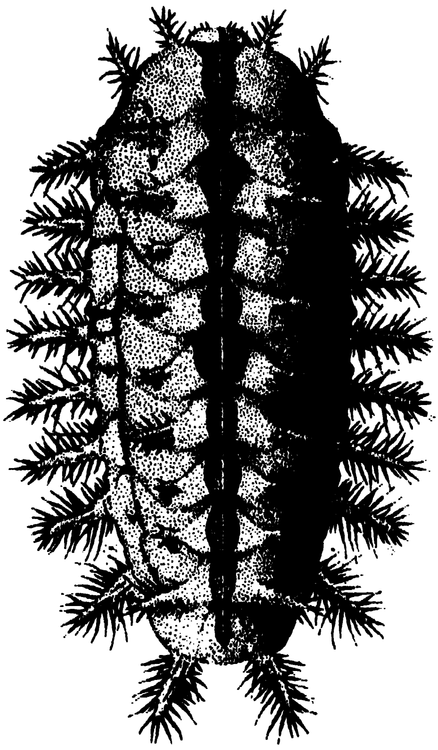
PLATE IV.



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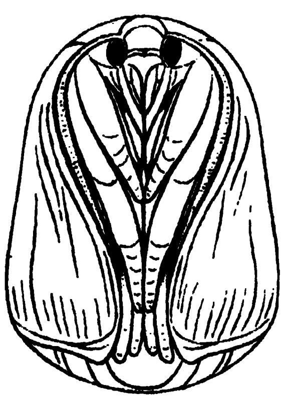

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PLATE V.

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PLATE VI.

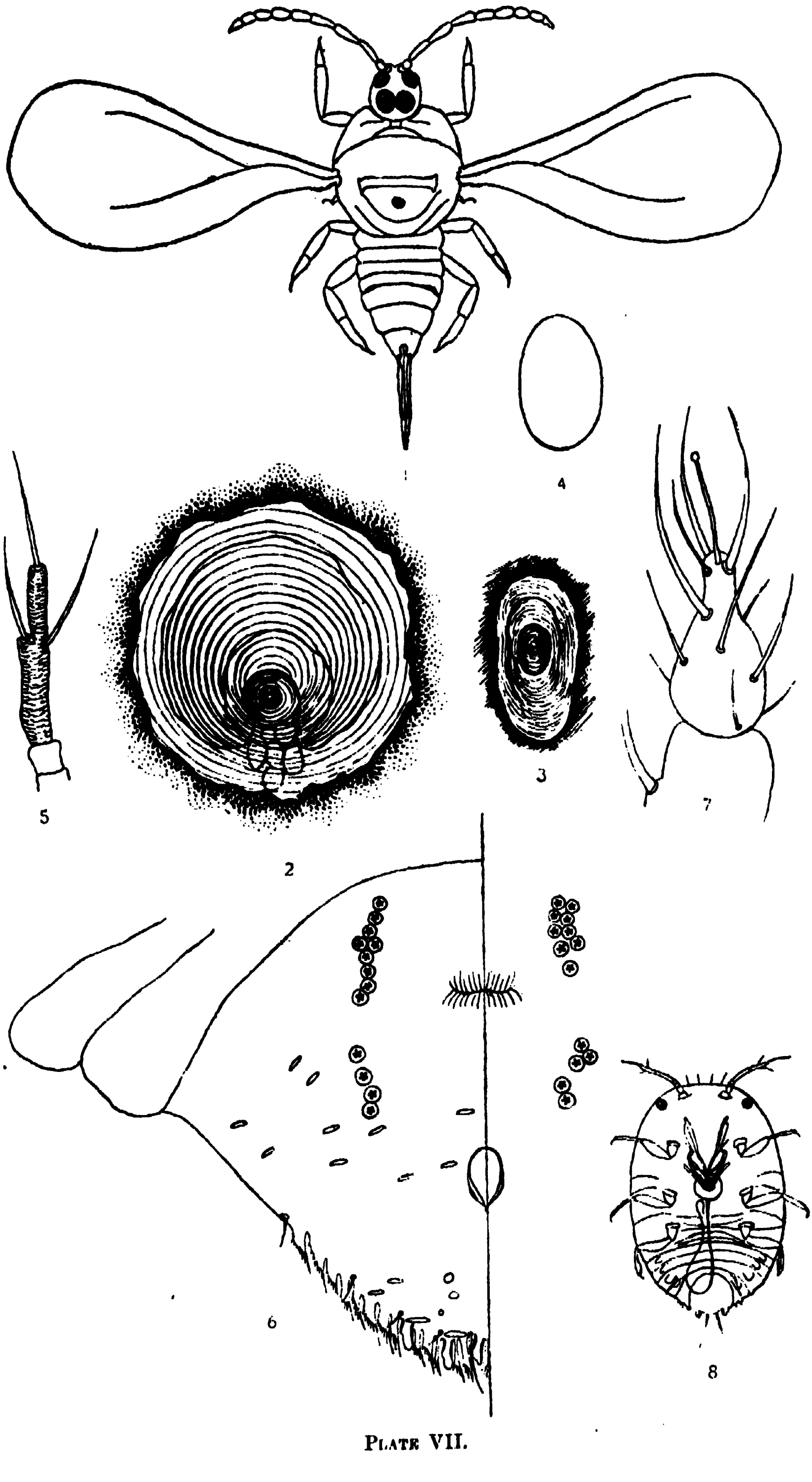

PLATE VII.

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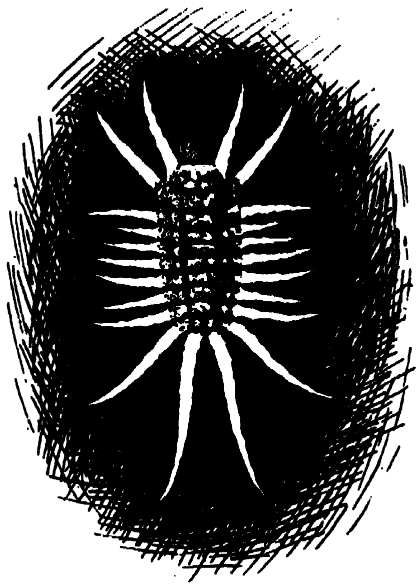

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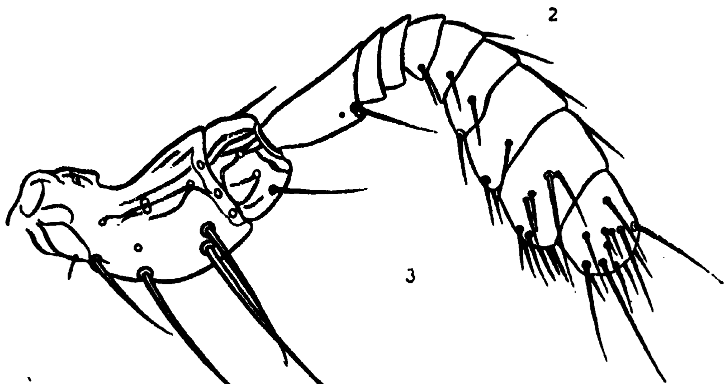

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PLATE VIII.

PLATE IX.

PLATE X.

NEW PHILIPPINE INSECTS.

By CHARLES S. BANKS.
(*From the Entomological Section of the Biological Laboratory, Bureau of Science.*)

The insects described as new in this paper were encountered while I was pursuing investigations upon the ones which injure the coconut palm. With one exception, that of *Thosea cinereamarginata* n. sp., they belong to the Coccidæ. Although twelve species of the Limacodidæ, including three of *Thosea*, are recorded from the Philippines, this number is small in comparison with those from neighboring regions, Hampson recording 82 species in his Fauna of British India.

LEPIDOPTERA.

LIMACODIDÆ.

Thosea cinereamarginata n. sp.

Thosea, *Walk. Cat.* (1855), **5,** 1068.
Aphendale, *Walk. Cat.* (1865), **33,** 494.
Anzabe, *Walk. Cat.* (1855), **5,** 1093.

Male: Head, thorax, and abdomen dark-brown; latter transversely banded dorsally with lighter brown. Two dorsal and anal tufts dark-brown. Tegulæ white-tipped. Fore-wing satiny dark-brown with purplish effulgence in certain lights. A moderately large darker spot at end of cell with a dark-brown oblique sinuate band beginning slightly posterior to it and extending to middle of inner margin; a similar, inward-curving, post-medial band beginning widely on costa before apex and extending nearly to posterior angle. Cilia attenuately spatulate, bronzed at tips and lighter than wing. Hind-wing grey-brown; veins and a discal spot slightly darker. Cilia slightly lighter. Under sides of wings grey-brown; fore-wing pearly at base and inner marginal area; hind-wing irrorated with dark-brown, scattered scales. Antennæ darker at base; first joint with long cilia. Palpi pale anteriorly. Tibiæ, tarsi, and venter with long, grey hairs. Length, 12 millimeters. Length of wing, 9.5 millimeters.

Female: Head, thorax, and abdomen brown-grey, latter transversely marked on apical dorsal margins of 5 to 7 segments with light ochraceous hairs. Fore-wing, except outer fourth, purplish brown-grey. Dark-brown spot at end of cell and an oblique dark-brown band as in male but much more distinct. Post-medial band parallel with outer margin for one-half its length, then curved inward and ending before posterior angle. Marginal area mouse-grey, irrorated with few, dark-brown scales. Cilia and hind wings as in male but latter with a light, clearly defined marginal line. Anal tuft with black-tipped hairs. Under side of wings

as in male. Legs with white-tipped, grey hairs. Antennæ and palpi dark-bronze brown. Length 12.5 millimeters, length of wing 11.25 millimeters.

Habitat: Manila (Banks Coll.).

On *Cocos nucifera* L.

Types of male and female: No. 4888 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

HEMIPTERA.

COCCIDÆ.

DIASPINÆ.

Chrysomphalus propsimus n. sp.

Chrysomphalus Ashm., *Am. Ent.* (1880), **3**, 268.

———————— *Orange Ins.* (1880), 21.

———————— Leon., *Riv. Pat. Veg.* (1899), **7**, 108.

Aonidilla Berl. e Leon., *Riv. Pat. Veg.* (1895), **4**, 77.

———— Idem. (1899), **7**, 174. Type aurantii.

s. g. Melanaspis Ckll, *Bul. T. s. U. S. Dep. Agr.* (1897), **6**, 5. Type, obscurus.

s. g. Mycetaspis Ckll. *Bul. T. s. U. S. Dep. Agr.* (1897), **6**, 5. Type, personatus.

Inaspidiotus Barreda, *Boll. Com. Parasit. Agr.* (1901), 229.

Chrysomphalus Fernald, *Cat. Coc. World* (1903), 285.

Type: ficus.

Female puparium (Pl. I, fig. 2) circular, convex, 1.5–2.5 millimeters in diameter, dark-chocolate brown, grayish at margin. Pellicles (Pl. II, fig. 2), dark-golden orange, covered with white, waxy film in perfect specimens; central or subcentral; a small tubercular prominence at center of first pellicle, surrounded by slight depression. Margins of pellicles lighter than disc, which is scabrous. Ventral scale exceedingly thin, white, waxy; often remaining intact upon leaf after upper scale has dropped off and giving the surface a moldy appearance. Male puparium (Pl. I, fig. 3), 0.75–0.90 millimeter long, obtuse-oval in outline; of same general color as female; pellicle slightly eccentric or near one extremity and dark-orange. Adult female (Pl. II, fig. 1): 1.05 millimeters long, 0.90 millimeter wide, nearly circular in outline, except posterior area, which is sharply prolonged to apex of pygidium; yellow; mesothoracic margin very slightly thickened and bearing a spine, minutely denticulate at its base. (Pl. II, fig. 4.) Pygidium (Pl. II, fig. 3) acute laterally; the 6 lobes subequal in size and each having an acute exterior tooth; squames deeply compound and fimbriate, 2 in mesal, first and second spaces, and 3 in third space beyond third lobe. Beyond last squames, margin is thickened and minutely denticulate, dentations in a continuous group, thus differing from *Chrysomphalus aonidium* Linn., to which the scale is most closely related. Margins of abdominal segments quite markedly lobed, each bearing a spine. Two spines at base of pygidium on each side. Circumgenital glands in 4 groups, the posterior with 4 and the anterior with 6 to 8 orifices. Genital aperture between posterior pair of glands. Dorsal pores of tubular segments in 2 subparallel series on each side, containing 18–23 orifices, extending halfway to base of pygidium. Entire margin of insect exterior to pygidium, minutely serrate, with slender spines at irregular intervals. Anterior spiracles opposite middle of rostrum, posterior halfway between rostrum and base of pygidium.

Adult male (Pl. I, fig. 1) 0.88 millimeter long, including genital style, very stout; wing 0.60 millimeter long, yellow; apodema darker; eyes crimson-red and occupying nearly entire head. Antennæ nearly as long as body, exclusive of the

style which is 0.27 millimeter long; antennal segments having long hairs; tarsal claw long, slender, spiniform with apex curved.

Eggs, yellow, 0.15 millimeter long.

Young: Yellow, 0.18–0.20 millimeter long, 0.13 millimeter wide.

Habitat: Philippines, Manila; San Miguel de Mayumo, Prov. of Bulacan (Banks Coll.).

On leaves of *Cocos nucifera* L., both surfaces.

Type: No. 10164 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species is so like *C. aonidium* L., that I hesitated to call it new, but the color of the puparia and pellicles of male and female and the difference in pygidial characters make the necessity for its separation as a new species quite evident.

Parlatoria greeni n. sp.

Parlatoria Targ., Catalogue (1869) 42.
——— Sign., *Ann. Soc. Ent. Fr.* (1869) (4) **9**, 450.
——— Green, *Coccidæ Ceylon* (1899), 162.
——— Fernald, *Cat. Coc. World* (1903) 318. *Et al. in litt.*

Type: lucasii = ziziphi.

Female puparium (Pl. III, fig. 1) pale to dark-slate color, lighter at margins; of a regular, broad-oval; 1.35 to 1.65 millimeters long, 0.90 to 1 millimeter wide. First pellicle broad-oval, narrow in front; of a transparent, yellow-ocher, having a broad, emerald-green, median, carinated band from front to hind margins. Second pellicle circular, except for squames at posterior extremity; of same color as first pellicle; with a broad, subtriangular emerald-green median band from anterior margin to disc and transverse, oval patch of the same color at posterior extremity. Pellicles at anterior extremity of puparium and occupying, in old specimens, slightly less than one-half total length. Their axes seldom in line with main longitudinal axis of puparium. Ventral scale a mere flange one-sixth the width of the puparium.

Male puparium (Pl. III, fig. 2) 0.87 millimeter long, 0.26 millimeter wide, of a dirty-white; sides parallel, carinate; posterior margin rounded, posterior area flattened. Pellicle similar to first pellicle of female, tipped forward at an angle of 45 degrees.

Adult female (Pl. III, figs. 4 and 4 A) (living specimen): Broadly elliptical in outline; head tapering; body broadest across posterior three-fourths. Length 0.60 millimeter, width 0.45 millimeter, somewhat smaller than second pellicle, beneath which it lies. Discal area swollen. Marginal area flattened. Margin minutely serrated. Abdominal segments quite distinct, less so at margin. Disc, dark-purple, marginal area lighter, almost white in certain points. Pygidium pale-ocher tinged with purple. Anterior pair of spiracles slightly posterior to line across base of rostrum and each two-thirds of distance from rostrum to margin; posterior pair, each at a point posterior to apex of rostrum, equal to length of latter and one-third distance from median line to margin. Skin transversely papilliate. Tip of rostrum ocher, base paler, nearly white, length of rostral setæ one and one-half times that of body. Margin bears on each side isolated spines not exceeding 6, and a spinous tubercle at a point opposite apex of rostrum.

Pygidium (Pl. III, fig. 6) evenly rounded posteriorly. Lobes and squames (Pl. III, fig. 5) of about equal length, tips of squames very slightly longer. Six principal lobes subequal in size, tridentate. Rudimentary lobes distinct, cuspid, one-half the length of principal lobes. Order of arrangement of lobes, squames, and spines from mesal space as follows: 1 squame, 1 lobe, 2 squames, 1 lobe, 1 squame,

1 spine, 2 squames, 1 lobe, 1 spine, 1 squame, 1 spine, 2 squames, 1 rudimentary lobe, 1 squame, 1 spine, 2 squames, 1 rudimentary squame, 1 rudimentary lobe, 1 squame, 1 spine, 1 squame, beyond which a series of modified cuspid lobes on margins of second and third abdominal segments. Marginal semi-lunar pores prominent, 9 on each side, one median; first 2 on each side subtend 2 squames, remainder not as wide as 2, but wider than 1 squame. A submarginal series of dorsal oval pores more numerous and thickly placed near base of submarginal area of pygidium. Circumgenital glands in 4 groups of which the posterior pair contain each 5 openings and the anterior 6. This is quite constant. Anal orifice submarginal on dorsum.

Adult male not known.

Eggs (Pl. III, fig. 3) dark purple 0.28 millimeter long and 0.15 millimeter wide, placed transversely beneath scale.

Habitat: Philippines, Manila (Banks Coll.).

On upper surface of leaves of *Cocus nucifera L.*

Type: No. 10105 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species is most closely related to *Parlatoria proteus* Curt., but also resembles *P. pergandii* Comst., from which it differs in the color of the scale, *P. pergandii* Comst. being of a light yellow, transparent hue, while *P. greeni* n. sp. is slate color. The fourth lateral (first rudimentary) lobes are more developed and have dentate margins and a sharp terminal cusp. The color of the second pellicle is constant and characteristic.

This species is named in honor of my fried, Prof. E. Ernest Green, Government Entomologist of Ceylon, whose indefatigable labors in the study of the Coccidæ are known and appreciated in all parts of the world.

Chionaspis candida n. sp.

Chionaspis Sign., *Ann. Soc. Ent. Fr.* (1869), (4) **9**, 442.

——— Fernald, *Cat. Coc. World* (1903), 203. *Et al. in litt.*

Type: salicis.

Female puparium (Pl. IV, fig. 1) snow-white, 2.35–2.50 millimeters long, 1.15–1.23 millimeters wide. Considerably dilated posteriorly; narrow anteriorly, the sectionary area usually reaching middle of second pellicle, but sometimes to its anterior margin. Surface smooth, somewhat glabrous and showing only transverse lines of growth. Pellicles pale, fulvous, second slightly darker posteriorly. First pellicle, one-half the length and one-third the width of second, which it overlaps for one-half its own length. An almost imperceptible carina on second pellicle. Length of second pellicle 0.77 millimeter.

Male puparium (Pl. IV, fig. 2) snow-white, 0.85–0.90 millimeter long, 0.38 millimeter wide, with ill-defined median and lateral carinæ. Surface woolly. Pellicle one-third length of puparium and of a very pale-fulvous.

Adult female (Pl. IV, fig. 3) length 1.25 to 1.75 millimeters, width 0.65 to 0.80 millimeter; pale-yellow, pygidium with brownish tinge posteriorly. Form, elongate oval. Antennæ with basal knob, cuspid internally; apical joint stout, fleshy, quite similar in shape and size to larger pygidial squames, submarginal, on anterior part of head. Entire surface of body minutely palpillate. Dorsum, posterior to a transverse line through apex of rostrum, marked by 2 submedian subparallel, linear stomata diverging posteriorly. Abdominal segments acutely lobed (Pl. IV, Fig. 3). Anterior spiracles having well-defined parastigmatic glands and removed laterally from apex of rostrum a distance equal to width of latter at base. Posterior spiracles slightly smaller than anterior and placed a

little before middle of body, about equidistant from each other and from the margin. Pygidium (Pl. IV, fig. 4) with circumgenital area marked by a horseshoe of snow-white secretion, open posteriorly. Median lobes projecting one-third their length from an obtuse posterior notch; their interior margins minutely dentate (usually 5 dentations). External cusp rounded; laterad of this a small tooth; a pair of spines between median lobes. Exterior to median lobe a spinose squame, a marginal pore, 2 lateral lobes, the larger dentate laterally, and their margins rounded. Exterior to lateral lobes, a squame, 2 marginal pores; on dentations of margin, a squame, 2 marginal pores, a squame, 2 marginal pores, a squame, a marginal pore and 6 squames, latter on third abdominal segment. Segmental areas quite well defined by plications and series of dorsal pores, the latter forming on each side 4 distinct series as follows: On second abdominal segment 8 submarginal; on third, 4 submedian and 8 submarginal; on fourth, 3 submedian and 5 submarginal; on pygidium, 2 submedian, 5 submarginal and 1 exterior to anterior group of circumgenital glands. Cylindrical ducts short and stout. Circumgenital glands arranged in 5 groups, the median with 8, the anterior lateral with 14–19 and the posterior with 14–16 openings. Anal opening directly above genital—i. e., both lie in same vertical plane.

Adult male not known.

Eggs pale-yellow 0.30 millimeter long and 0.15 millimeter wide. Plainly visible through body wall of female.

Larvæ (Pl. IV, fig. 5) pale-yellow, 0.40 millimeter long upon emerging from egg.

Habitat: Philippines, Manila (Banks Coll.).

On upper surface of leaves of *Cocos nucifera* L.

Type No. 10102 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

Lepidosaphes mcgregori n. sp.

Lepidosaphes Shimer, *Tr. Am. Ent. Soc.* (Jan, 1868), **1**, 373.
Mytilaspis Sign., *Ann. Soc. Ent. Fr.* (1868), (4) **9**, 91; not described.
Phaulomytilus Leon., *Riv. Pat. Veg.* (1897), **6**, 205, 206.
Coccomytilus Leon., *Riv. Pat. Veg.* (1897), **6**, 205, 206.
Trichomytilus Leon., *Riv. Pat. Veg.* (1897), **6**, 205, 206.
Lepidosaphes Fernald, *Cat. Coc. World* (1903), 304.

Type: conchiformis=ulmi.

Female puparium (Pl. V, fig. 1) 2.50–2.65 millimeters long, 0.60–0.75 millimeter wide; very long and narrow, with the sides gradually diverging posteriorly and well rounded dorsally; clear, red-brown, with lighter to whitish margins; transverse striæ separated into groups by more pronounced carinæ. First pellicle broadly oval, yellow-ochraceous, with broad, median carina and 2 waxy, anterior horn-like projections; second pellicle slightly darker, more elongate, narrowing abruptly and acutely posteriorly, hind margin and an anal patch light ochraceous, with a transverse discal carina and a decided spine on margin of second abdominal segment.

Male puparium (Pl. V, fig. 2) 1.45 millimeters long, 0.35 millimeter wide, narrow anteriorly and widening behind; posterior margin subcircular. Pale, yellow-brown anterior, waxy, horn-like projections lacking; posterior and lateral margins narrowly white; a transverse, pre-anal, thin portion of scale marking portion which may be elevated for escape of adult male.

Adult female (Pl. V, fig. 5) 0.90–0.95 millimeter long, 0.35 millimeter wide, of a light-yellow, almost white; the anterior extremity slightly narrower, widest part just anterior to pygidium. Antennæ halfway between front margin and base of rostrum and having each an axillary spine mediad, nearly equal to them

in length. Anterior spiracles opposite apex of rostrum and halfway to lateral margin, with a few stigmatic orifices anteriorly. Mentum subtriangular. Posterior spiracles conspicuous. Abdominal segments easily differentiated and strongly lobed at margins; first segment spineless, second with 3, third and fourth with 4 spines each. Thoracic cuticle not especially chitinous; irregularly papilliate. Pygidium (Pl. VI, fig. 3) narrower than long, pale-ochraceous, slightly darker than abdominal margins. Anal orifice three times its own diameter, from anterior margin of pygidium, protected on each side by a longitudinal fold, the 2 converging posterior to anus and exteriorly to disc of pygidium in parallel lines. Circumgenital glands in 5 groups, the median with 4 orifices, the anterior lateral with 6 and the posterior with 5 to 6. The grouping is not well defined. Median lobes broad, their surfaces crenulate, rounded, and having a distinct tooth at each side; second pair of lobes double, their margins straight. A median, acute tubercle in mesal space, with a long spinous squame on either side; 2 squames between first and second double lobes; 2 beyond second double lobes, 2 groups of 2 each toward base of pygidium. Margin of pygidium beyond lobes thickened and minutely serrated, with 3 prominent teeth, in the first 2 of which are 2 oval, glandular orifices, in the basal of which, one. Another similar orifice between median and second lobes, making total of 6 for each side. Genital orifice one-third distance from median group of circumgenital glands to posterior margin. Numerous, circular, dorsal pores irregularly arranged, 2 being submedian posteriorly, 1 below each of second lobes and remainder near center of disk.

Adult male not known.

Eggs (Pl. V, fig. 3) 0.28 millimeter long, 0.11 millimeter wide, of a rather light purple, very regularly arranged in puparium (Pl. V, fig. 4).

Larva (Pl. VI, fig. 2) 0.29 millimeter long, 0.11 millimeter wide, slendér, somewhat polygonal, bright-yellow, having a prominent spine on posterior, lateral margin of mesothorax. Antennæ slender, transversely striate, with few hairs, 2 stout ones at tip; a few spines on posterior and anterior extremities, 2 submedian caudal setæ one-third length of body. Tarsal knobbed hairs twice length of ungues.

Habitat: Philippines, Manila (Banks, Coll.).

On both surfaces of leaves of *Cocos nucifera* L.

Type: No. 10142 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species differs from *L. cocculi* Green in having no cephalic transverse fold, in the ventral scale being incomplete, in the color and disposition of the eggs, and in the form of the median lobes. In addition, the general appearance of the scale and the perceptible prolongation of the anterior, waxy, horn-like projections readily differentiate it as a separate species.

I take pleasure in naming this species for my friend Richard C. McGregor, a most diligent collector and an accurate observer of insect life in the Philippines.

Lepidosaphes unicolor n. sp.

Female puparium (Pl. VII, fig. 1) 1.80–1.90 millimeters long, 0.50 millimeter wide, of about the same general shape and size as *L. mcgregori* n. sp., except that fore end of first pellicle is more acute and marginal flattening is hardly perceptible; of a dark-red or dark-cherry-brown including both pellicles, except a lighter patch on sides and caudal end of second. Both pellicles with median carina, puparium transversely multicarinate (usually 7 carinæ), the carinæ sep-

arating transversely-striate, paler areas. At posterior end there is a pair of subparallel, longitudinal elevations or carinæ. This feature appears to be constant.

Male puparium not known.

Adult female (Pl. VII, fig. 3) 0.90 to 0.05 millimeter long, 0.35 millimeter wide, of a pale, transparent, cream-color, nearly white, narrow anteriorly, widening uniformly to third abdominal segment, then somewhat abruptly rounding to extremity. Front rounded; latero-frontal angles of head produced laterally into spinose, concentrically papilliate knobs. (Pl. VII, fig. 4.) Antennæ nearer front than base of rostrum; secondary spine as long as primary and usually strongly curved. First spiracles opposite mentum, nearer margin. Abdominal segments strongly produced laterally, then posteriorly. The second, third, and fourth with 3 to 4 stout spines and the second with a lateral cusp. Pygidium pale-ochraceous. Circumgenital glands hardly separated into groups, but consisting of an anteriorly curving series of 24 orifices, the median 4 of which are slightly separate from the laterals. Anal orifice a little more than its own diameter from base of pygidium; genital one-third the distance from the median group of glands to caudal margin. Median lobes as in *L. mcgregori* n. sp. Second double lobes with margins more rounding. Squames pseudo-fimbriate, the single fimbriation indicated on each by mere acute tubercle. (Pl. VII, fig. 6.) A median acute tubercle is present between mesal squames. Marginal orifices, on each side, with cylindrical spinnerets long and narrow; neither as large as in *L. mcgregori* n. sp. On dorsal area of pygidium are irregularly placed oval discal pores which are quite prominent in all specimens.

Adult male not known.

Eggs (Pl. VII, fig. 5) snow-white, 0.215 millimeter long, 0.10 millimeter wide, somewhat conical in form and laid in regular rows beneath puparium.

Larvæ (Pl. VII, fig. 7) pale-yellow, 0.23 millimeter long, 0.11 millimeter wide. Rather stout oval; front with 4 curved setæ, mentum circular, antennæ moderately stout, setose. Apices of tibiæ larger than bases; caudal margin with 6 spines on each side.

Habitat: Philippines, Manila (Banks, Coll.).

On upper surfaces of leaves of *Cocos nucifera* L.

Type: No. 10171 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This scale is quite distinct in appearance from *L. mcgregori* n. sp. Its color is uniform and the transverse carinæ are fewer and less prominent on the puparium. The 4 irregularly placed discal pores of pygidium are not present in *L. mcgregori* n. sp. The great length and shape of the lateral abdominal margins and the shape of the squames serve to differentiate this as a new species.

Paralecanium cocophyllæ n. sp.

s. g. Parelecanium Ckll. & Parr., *The Industrialist* (1899) 277.
———— Ckll., *Can. Ent.* (1901) **33**, 57.
g. ———— Ckll., *Ann., Mag. Nat. Hist.* (1902) (7) **9**, 455.
———— Fernald. *Cat. Coc. World* (1903) 199.

Type: frenchii.

Adult female (Pl. VIII, fig. 1) 4–5 millimeters long, 3.5 4 millimeters wide; of a broad oval, almost circular and very slightly narrower anteriorly; margin with shallow, obtuse notches at stigmatic and anal clefts. Of a pale, transparent yellow; a distinct marginal area which is applied to leaf and which has its interior border marked by a thin, golden line. An orange-yellow patch on each

side of posterior area marking position of ventral egg cavities. These cavities lined with an orange-colored, viscid substance which soon dries upon exposure. A submarginal translucent area and a similar median one through which movements of internal organs are visible. An irregular, broken line of dark-brown spots, internally, shows through dorsal wall and probably marks lower part of alimentary canal. Dorsal surface minutely punctuate, and covered with a very thin, waxy substance in addition to a well-defined, longitudinally curved series of triplicate, polygonal, waxy laminæ, the smaller of each of which is uppermost. These laminæ are arranged as follows: A median series of 6 (sometimes the posterior is divided to form a seventh), a submedian of 9, a submarginal of 13 to 16 and a marginal of 28 to 30, on each side. (Pl. VIII, fig. 1.) The laminæ mark the insect's stages of growth. (Pl. X, fig. 4.) Regularly arranged suboval pores over entire dorsum (Pl. VIII, fig. 2), but on each side of anal plate there is an outwardly and anteriorly curving series of 4 groups of pores. (Pl. VIII, fig. 7.) Marginal area with reniform, radiating cells arranged like scales on a fish. Anal opercular scales (Pl. VIII, fig. 5) triangular, pointed; the free edge being about equal to or slightly longer than base. Stigmatic clefts circularly incised at margin and invariably with 3 long, stout, blunt, curved spines (Pl. X, fig. 2), these spines not attaining outer margin, being removed their own length therefrom. Margin with series of flabelliform scales slightly overlapping (Pl. VIII, fig. 4), which are wider in proportion to their length than those of *P.* (*Lecanium*) *expansum* Green. Between the bases of flabellæ, the margin is minutely dentate, the number of dentations being nonuniform. Eyes, red-brown, shining, placed two-thirds distance from rostrum to margin. Antennæ (Pl. VIII, fig. 3) indistinctly 3 jointed, placed nearly in a line with and between rostrum and eyes at 3 times their own length from latter. Legs wanting. Genital orifice (Pl. VIII, figs. 5 and 6) obovate, anterior to anal aperculum at a distance equal to length of latter; containing an interior series of small and an exterior of large glands or pores. Exterior to orifice on ventrum minute spinnerets in 4 ill-defined groups on each side.

Male puparium (Pl. IX, fig. 2) 2.27 millimeters long, 1.20 millimeters wide, elongate-oval, silicious or waxy in structure, more convex than female and consisting of 17 plates, the posterior median being double the length of the anterior, thus differing from that of *P. expansum* Green in which the posterior median plate is divided, forming 2, or a total of 18. Upon each plate a series of widely separated waxy laminæ, indicating larval covering. (Pl. X, fig. 4.)

Adult male (Pl. IX, fig. 1), length, including genitalia, 1.55 millimeters; length of wing 1.18 millimeters, of a uniform, pale yellowish-ocher, apodema darker-ocher. Eyes bright crimson. Antennæ somewhat thickly and evenly setose from base to apex and with 3 knobbed hairs on last segment. Nervures of wings slightly darker than membrane. Legs as setose as antennæ; tarsal claw acutely conical.

Eggs (Pl. XI, fig. 2) pale-yellow, 0.38 millimeter long.

Larvæ (Pl. XI, fig. 1) of same color as eggs, 0.48 millimeter long, 0.26 millimeter wide, the caudal setæ one-seventh length of body. Eyes brown-red, marginal. Anal cleft strong and deep (Pl. X, fig. 1.)

Habitat: Philippines, Manila (Banks, Coll.).

On under sides of leaves of *Cocos nucifera* L. in fair abundance.

Type: No. 10141 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species differs from *P. expansum* Green chiefly in the color, the shape of the flabellæ, and the number of spines in stigmatic cleft, in the female, in the number of plates in the male puparium and the color of the apodema of the male.

ILLUSTRATIONS.

[All figures are highly magnified; exact size is indicated in descriptions.]

PLATE I.

FIG. 1. *Chrysomphalus propsimus* Banks, adult male.
2. Female puparium.
3. Male puparium.

PLATE II.

FIG. 1. *Chrysomphalus proposimus* Banks, adult female.
2. First and second pellicles.
3. Pygidium of female.
4. Lateral spine of female.
5. Egg.

PLATE III.

FIG. 1. *Parlatoria greeni* Banks, female puparium.
2. Male puparium.
2 A. Lateral view showing angle at which pellicle is inclined.
3. Egg.
4. Adult female.
4 A. Lateral view.
5. Lobe and squame of pygidium.
6. Pygidium of female.

PLATE IV.

FIG. 1. *Chionaspis candida* Banks, female puparium.
2. Male puparium.
3. Adult female.
4. Pygidium of female.
5. Larva immediately after hatching.

PLATE V.

FIG. 1. *Lepidosaphes mcgregori* Banks, female puparium.
2. Male puparium.
3. Egg.
4. Under surface of female puparium showing adult, eggs and eggshells; latter toward posterior extremity and closely compacted.
5. Adult female.

PLATE VI.

FIG. 1. *Lepidosaphes mcgregori* Banks, second pellicle.
2. Larva immediately after hatching.
3. Pygidium of female.

Plate VII.

Fig. 1. *Lepidosaphes unicolor* Banks, female puparium.
2. First and second pellicle.
3. Adult female.
4. Frontal margin of female, showing antennæ and antero-lateral prominences or knobs.
5. Egg.
6. Pygidium of female.
7. Larva, immediately after hatching.

Plate VIII.

Fig. 1. *Paralecanium cocophyllæ* Banks, adult female.
2. Magnified portion of derm, showing irregularly oval cells.
3. Antenna of female.
4. Marginal flabellæ.
5. Genital orifice and anal operculum.
6. Genital orifice, highly magnified.
7. Operculum of female showing curving series of pellucid glands extending toward margin.

Plate IX.

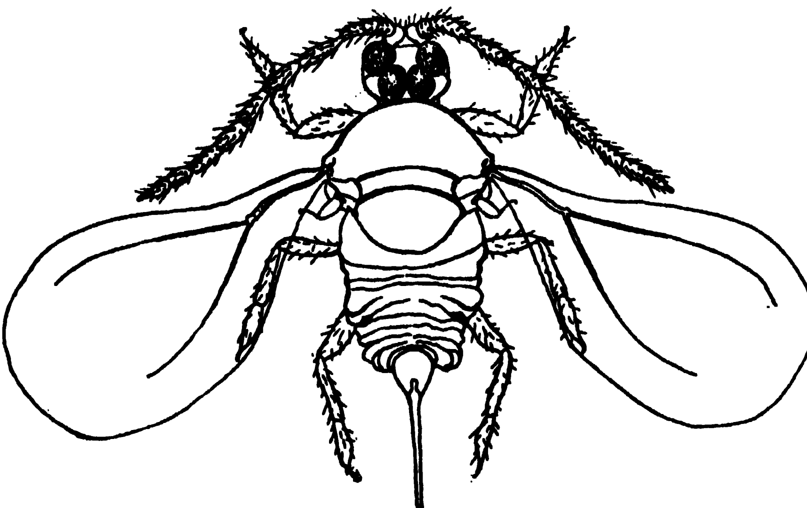
Fig. 1. *Paralecanium cocophyllæ* Banks, adult male.
2. Male puparium showing superposed laminæ.
3. Male pupa.

Plate X.

Fig. 1. *Paralecanium cocophyllæ* Banks, caudal extremity of larva.
2. Stigmatic cleft of female with spines and marginal flabellæ.
3. Mouthparts of female.
4. A single division or plate of male puparium showing dorsal laminæ of larva, separated by growth of the insect. The sides which were formerly proximate are numbered from 1 20.

Plate XI.

Fig. 1. *Paralecanium cocophyllæ* Banks, larva immediately after hatching.
2. Egg.
3. Antenna of larva.
4. Anterior leg of larva.
5. Middle leg.
6. Posterior leg.



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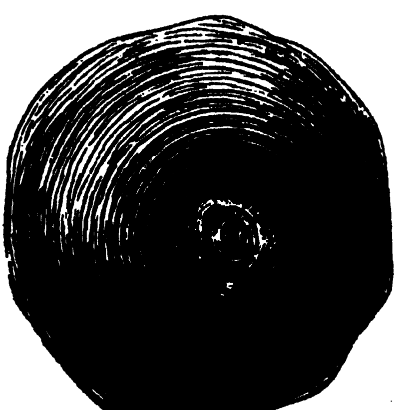

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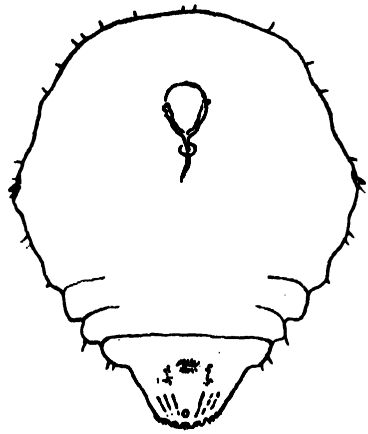

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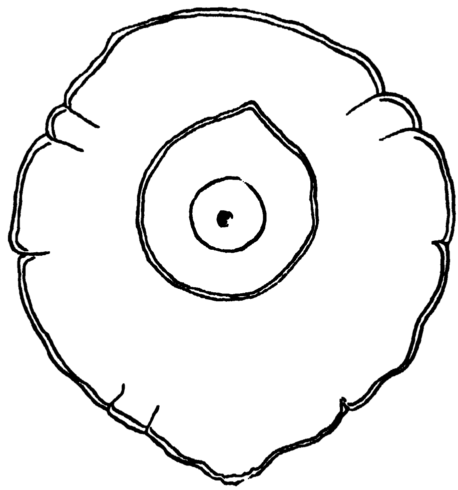

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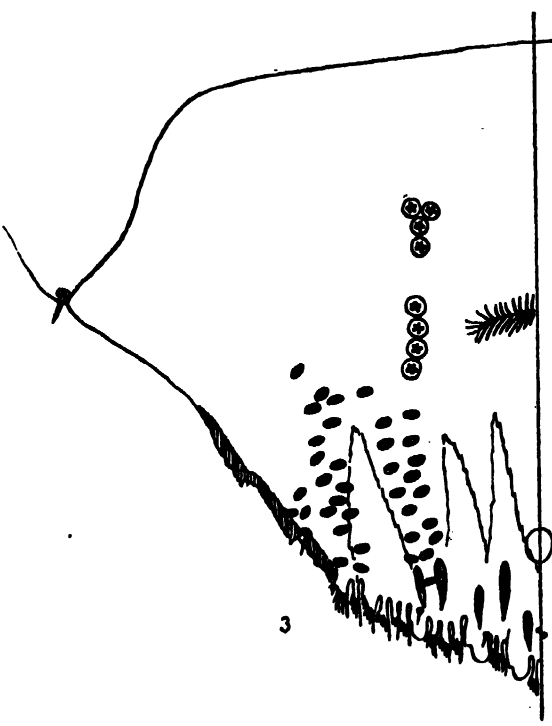

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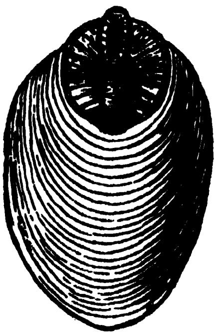

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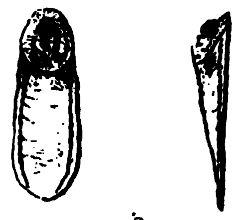

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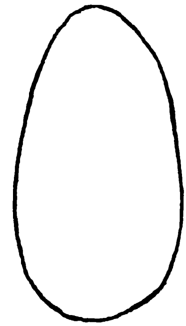

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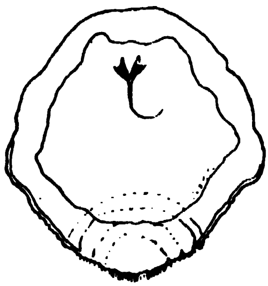

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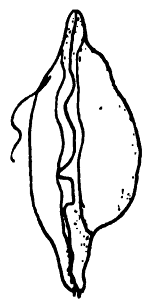

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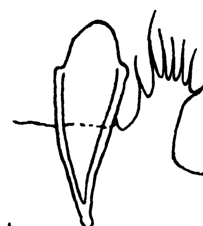

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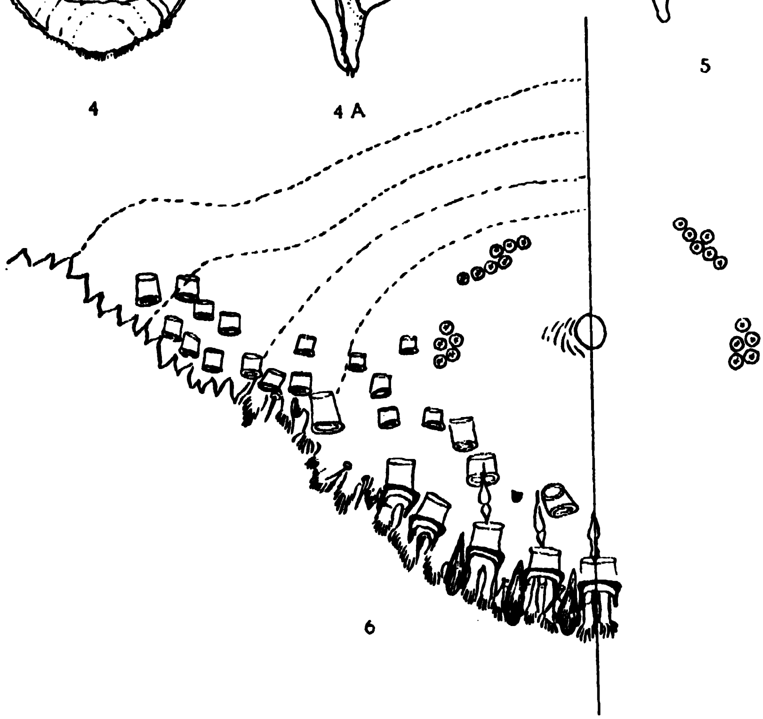

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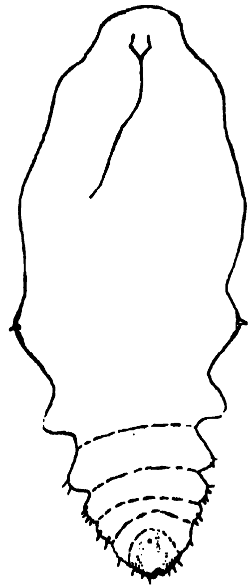

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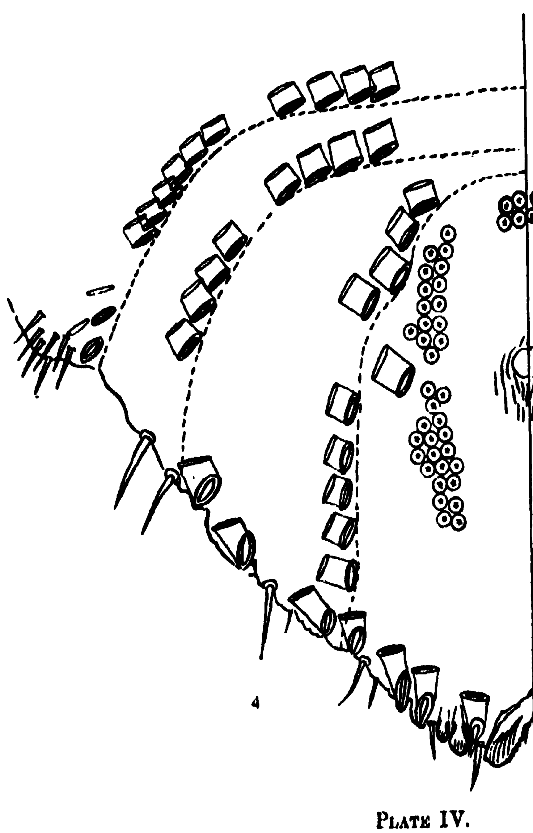

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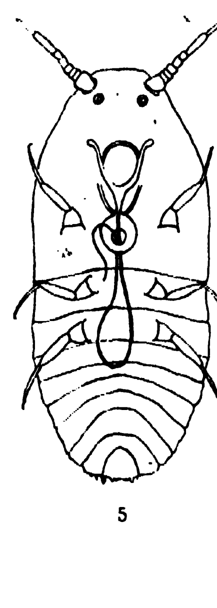

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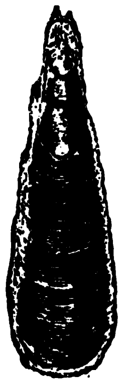
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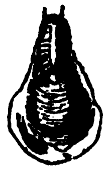
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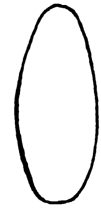
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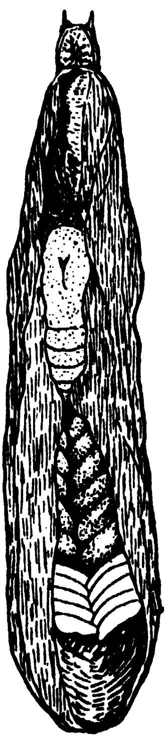
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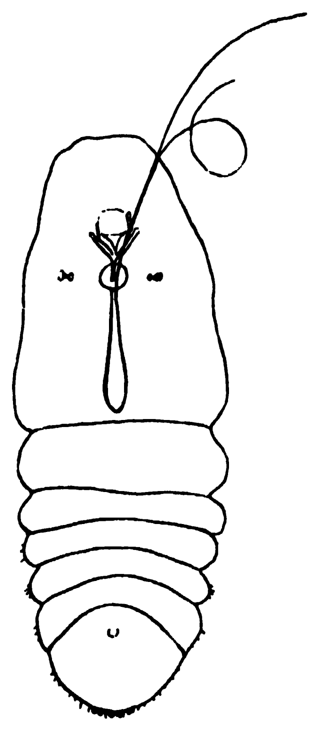
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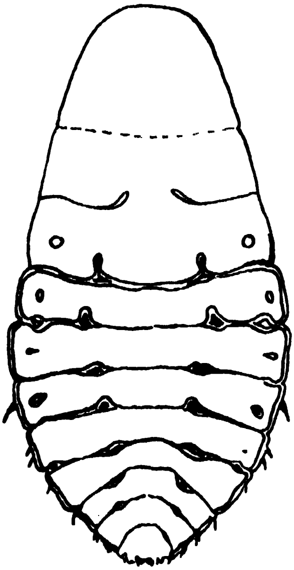

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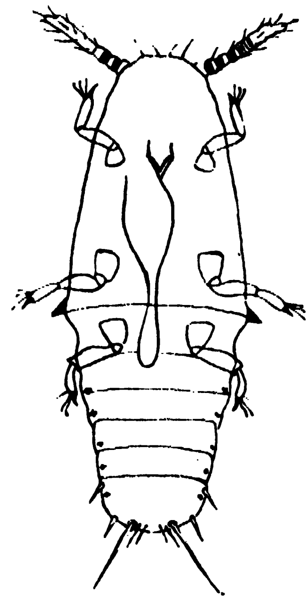

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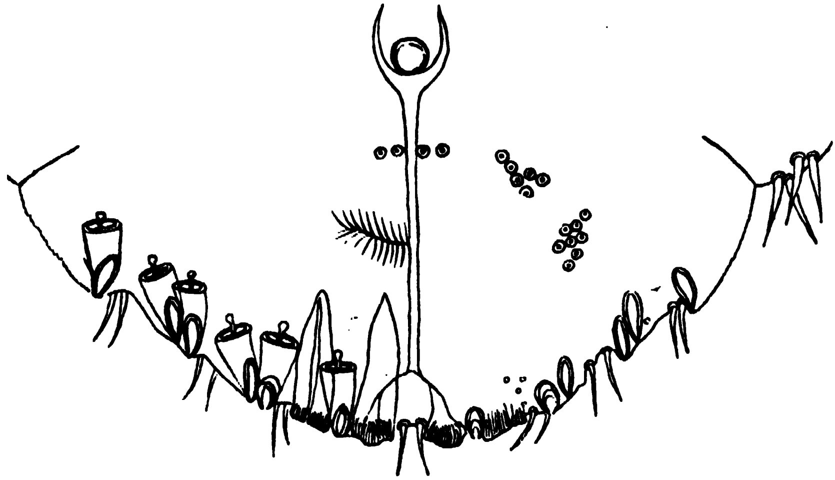

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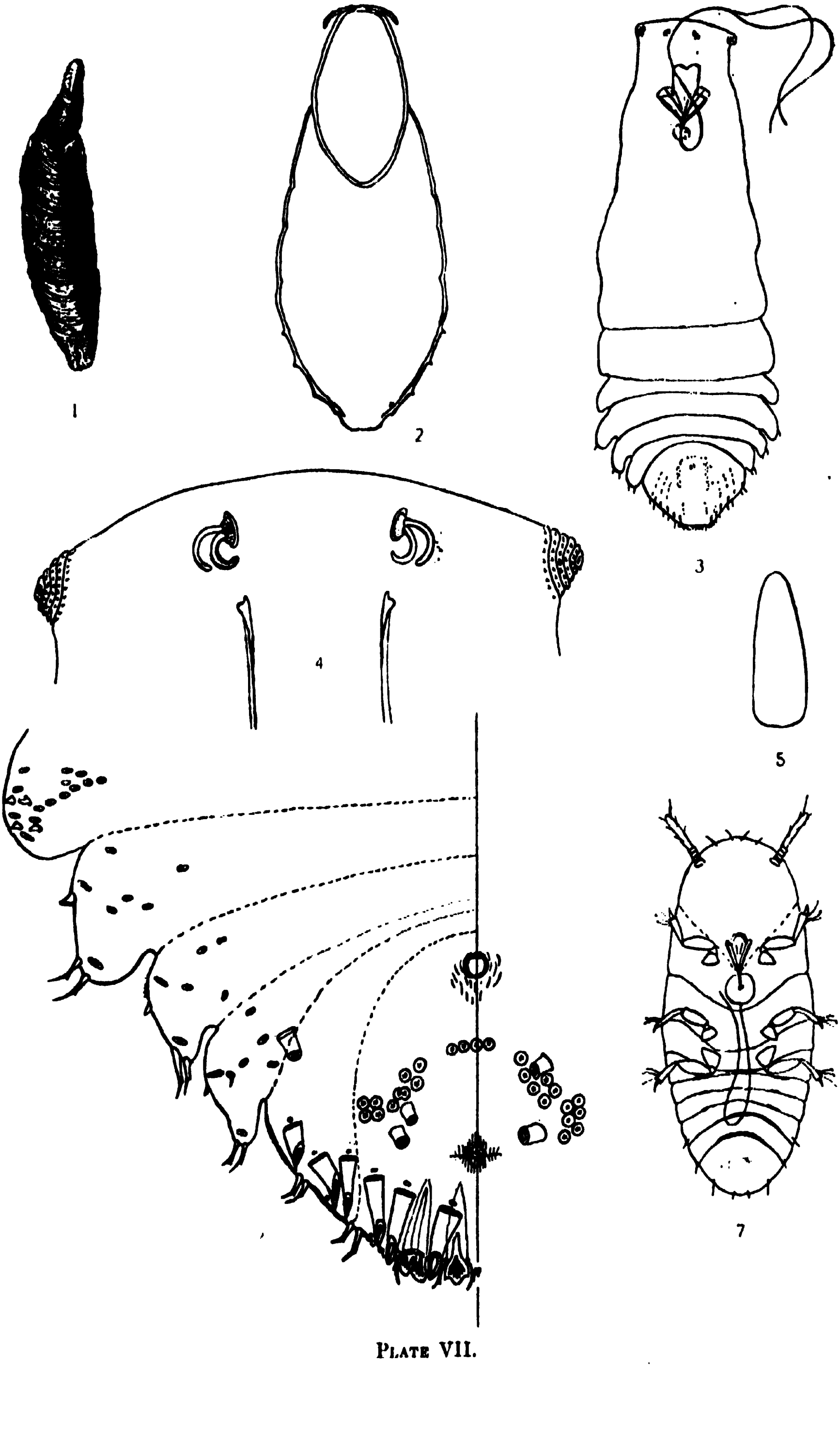

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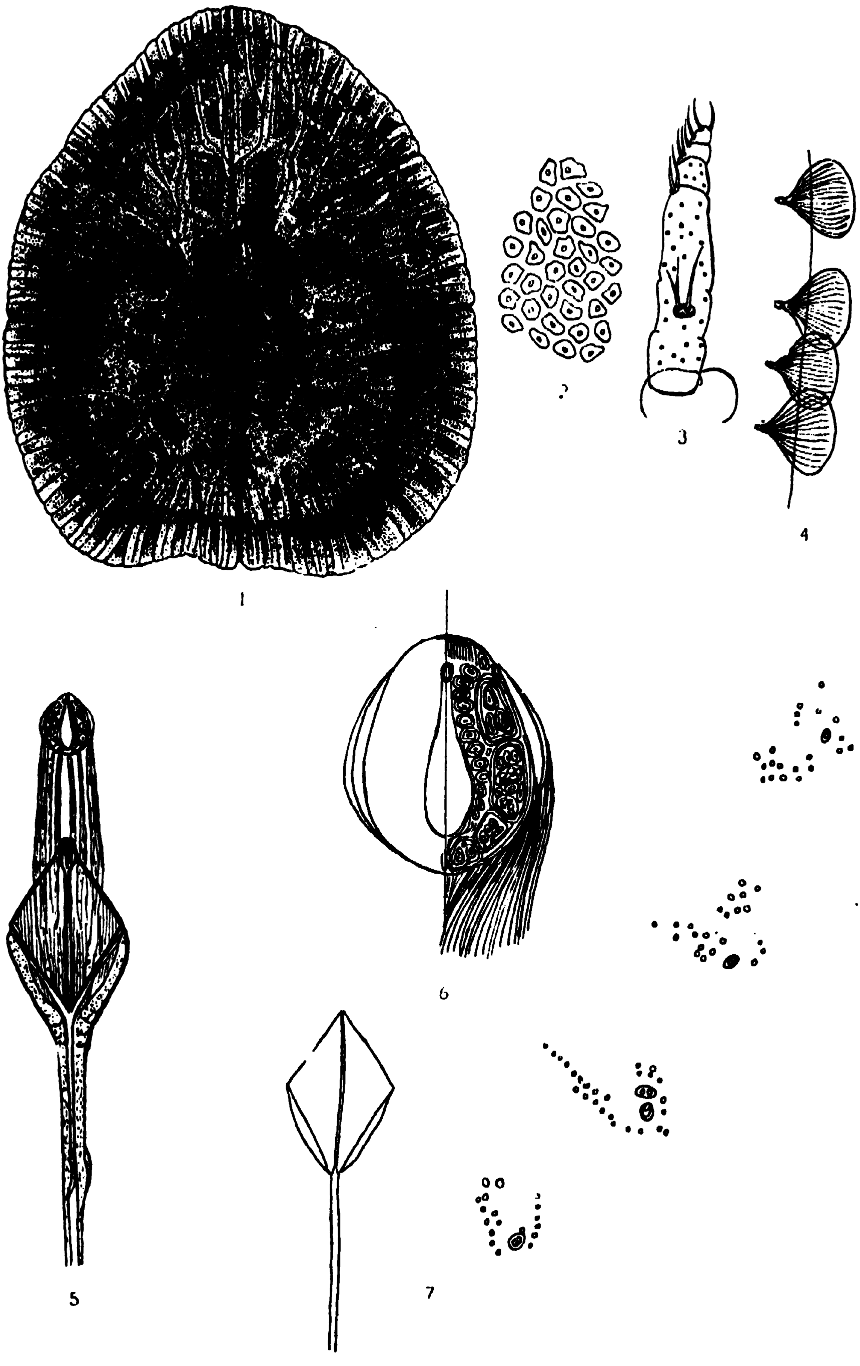

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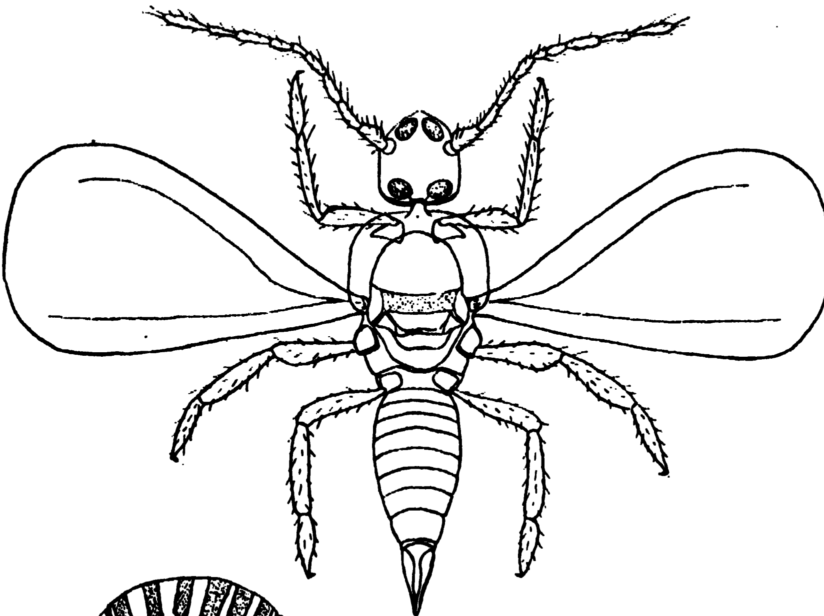

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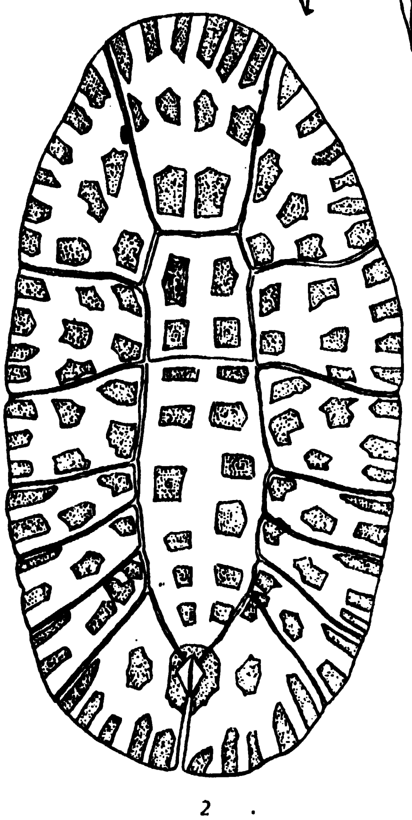

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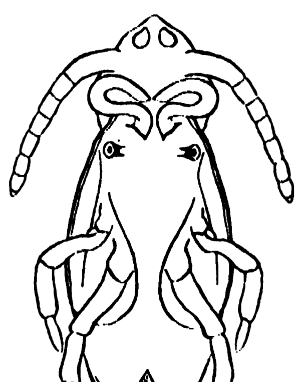

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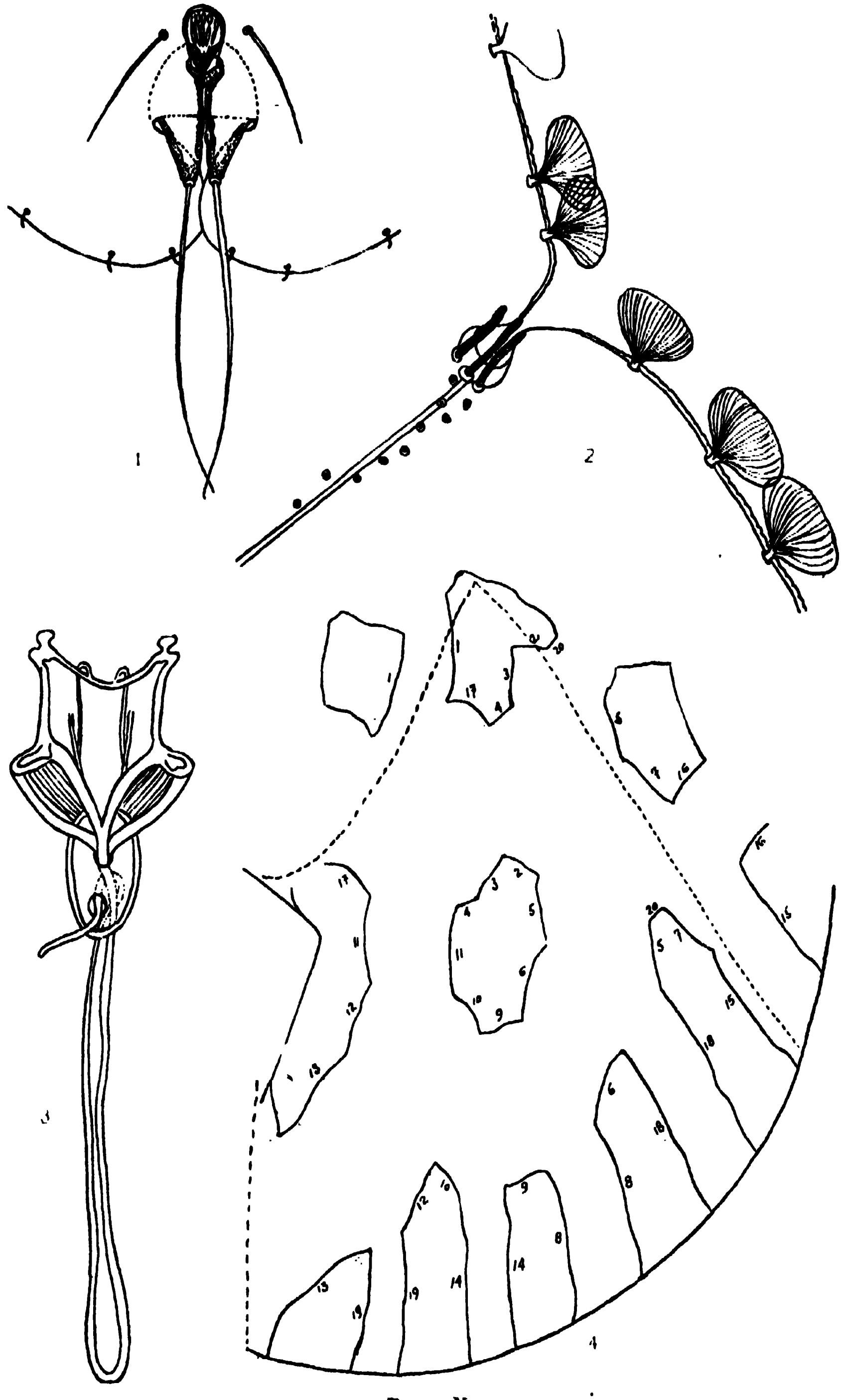

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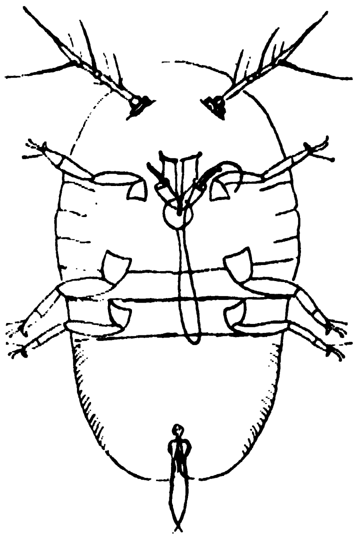

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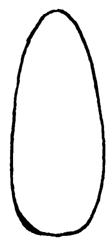

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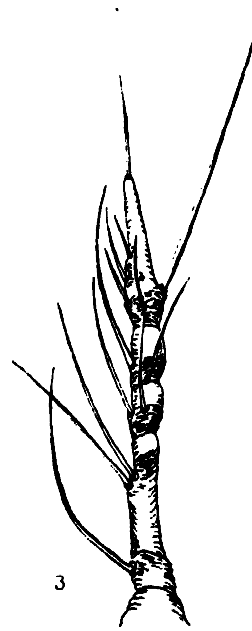

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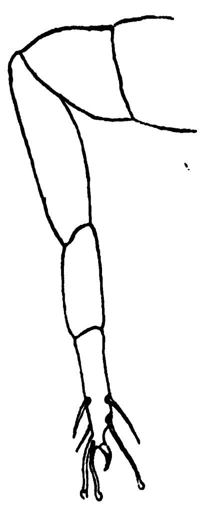

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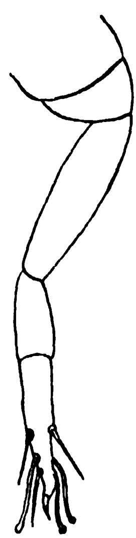

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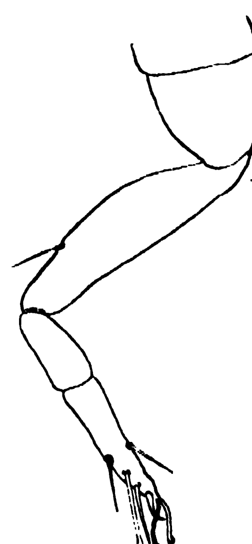

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PLATE XI.

STUDIES UPON EXPERIMENTAL VARIOLA AND VACCINIA IN QUADRUMANA.

By WALTER R. BRINCKERHOFF and E. E. TYZZER, with an introduction by W. T. COUNCILMAN.

(*From the Sears Laboratory of Pathology, Harvard University Medical School, Boston, and the Biological Laboratory, Bureau of Science.*[1])

CONTENTS.

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INTRODUCTION.

The investigation of smallpox is attended with difficulties. The disease only appears at intervals. The cases are not treated in well-organized hospitals where there are facilities for and the habit of the investigation of diseases. As a rule the hospital in which the disease is treated are used only at intervals and are unprovided with laboratories. Clinical teaching, the great stimulus to research, has no place in them. The energies of the physician in charge are entirely taken up in controlling the exigencies of an unusual situation. This isolation of the disease is unfavorable in that the valuable aid given by constant comparison with other diseases is lost.

Difficulties also attend the experimental study of smallpox. Such study can not be carried out in ordinary laboratories, owing to the fear of infection extending from the laboratory.[2] The ordinary laboratory animals such as rabbits, guinea pigs, and all the domestic animals are immune to the disease; the only animal, so far as is known at present, which is susceptible is the monkey. These are expensive, difficult to acquire, and in this climate very susceptible to disease. The majority of monkeys obtained from animal dealers are infected with tuberculosis.

During the epidemic of smallpox which prevailed in Boston in 1901 and 1902, an investigation of the disease was undertaken by members of the pathological department of the Harvard Medical School. The health authorities of the city gave every facility for investigation which was possible. Autopsies were held on 52 cases, embracing all forms of the disease and provision was made enabling certain of the investigators to live in the smallpox hospital and there to undertake some experimental work.

In the course of this investigation it was found that certain cell inclusions, first described by Guarnieri, were constantly associated with the lesions of both vaccinia and variola. These bodies are not of invariable form, but they show a series of forms corresponding to the developmental phases of a living organism. In the course of this development a body much larger and more complicated in structure follows the smallest and simplest forms, which body finally segments into a number of small,

[2] It is remarkable how persistent is this fear of smallpox. That it so persists is an evidence of the horrors of the disease in the prevaccination period. There is no disease which is so feared by the community as is smallpox, and certainly none against which we have such perfect protection.

simple forms corresponding to the ones which are the first to appear in the lesions. In variola, in addition to the forms common to both vaccinia and variola which are found in the cytoplasm of the epithelial cells, a new body appears in the nuclei of the epithelial cells, which undergoes a development dissimilar to that of the cytoplasmic forms, and which finally results in the formation of a sporoblast with spores.

It was believed by the investigators that these inclusions were living parasites and that in both vaccinia and variola there was a simple development taking place in the cytoplasm of the epithelial cells. In smallpox there was a further development which took place within the nucleus and which terminated in the formation of spores, which spores constituted the contagium of smallpox. The material was further worked over by Prof. G. N. Calkins, of Columbia University, who described a life history embracing both the cytoplasmic and intranuclear forms. It was further established that when vaccine virus was inoculated in a susceptible animal (and most animals are susceptible) a typical lesion was produced in which only the cytoplasmic forms of the organism were found. The same was true when variola virus was inoculated in animals not susceptible to variola. No exanthem develops on such animals after inoculation with variola virus. When variola virus was inoculated on the monkey an exanthem analogous to that of variola in man was produced and in both the primary lesion and in the exanthem the cytoplasmic and the intranuclear forms of the parasite were present.

The investigation in Boston was brought to a close by the lack of cases and the difficulties of experimental work. It had been carried to a point where further experimental work was necessary to secure all phases of the disease variola, to study further the interrelation between variola and vaccinia and the immunity problems involved. For these purposes the extensive use of monkeys as experimental animals was indispensable.

To secure experimental material, the best conditions for laboratory work, with access to variola in man to obtain fresh virus, the Biological Laboratory in Manila seemed to offer the best facilities. The proposition was favored by the United States Philippine Commission, who gave us every facility for the prosecution of the work, and Drs. W. R. Brinckerhoff and E. E. Tyzzer, who had taken a prominent part in the investigation of smallpox and vaccinia in Boston, were sent to Manila. To the Hon. Dean C. Worcester, Secretary of the Interior of the Philippine Islands, we wish to express our appreciation and thanks for his numerous efforts in our behalf. Dr. Paul C. Freer, Director of the Bureau of Science,[3] took much interest in our work and essentially furthered it. Dr. Richard P. Strong, Director of the Biological Laboratory, Bureau of Science, in which we worked, was a constant support to us in our work and did much to make

[3] Since the completion of the experimental work detailed in this paper, the Bureau of Government Laboratories of the Philippine Islands has been increased in its scope and its name has been altered to The Bureau of Science.—EDITOR.

our stay agreeable as well as scientifically successful. We wish also to extend our thanks to the members of the laboratory staff, especially to Dr. Wm. E. Musgrave, Pathologist, Dr. Wm. B. Wherry, Bacteriologist, and Dr. Maximilian Herzog, Pathologist, for many favors.

The constant supply of fresh variola virus requisite for our work was secured through the kindness of Maj. E. C. Carter, Commissioner of Public Health, and of Dr. V. G. Heiser, Chief of the Quarantine Service. A constant supply of fresh vaccine virus was given us by Dr. Paul G. Woolley, chief of the Serum Laboratory, Bureau of Science. Dr. W. R. Moulden, resident physician at Bilibid Prison, and Dr. H. B. Wilkinson, resident physician at the San Lazaro Hospital, gave us opportunity to study the clinical material under their control.

The greater part of the funds which made the expedition possible was generously supplied by Mr. Augustus Hemenway, Dr. John C. Phillips, and Dr. Wm. L. Richardson. The Rockefeller Institute for Medical Research aided the expedition by appointing one of the workers to a research fellowship.

If material taken from a smallpox lesion on man be inoculated on an epithelial surface of a calf, after a definite period, a lesion which anatomically closely resembles the parent lesion, the pock is produced. Its appearance is accompanied by swelling of the nearest lymph nodes, fever and constitutional disturbance. After the process has subsided there is immunity to further inoculation. The material from the lesion, transferred to an epithelial surface on another calf, produces a similar result, and after a series of transfers from animal to animal may be returned to man, and it develops not the original disease, smallpox, but the incomparably milder disease, vaccinia.

Many of the strains of vaccine virus now used are known to have been derived from smallpox and we are justified in believing that all strains were originally so derived. Just how many transfers from animal to animal is necessary before the virus loses its power to produce smallpox is not known. One of our experiments in this regard is interesting. The contents of a smallpox vesicle in a monkey was used to inoculate the cornea of a rabbit. After 5 successful transfers to other rabbits the virus was used to inoculate a monkey and not vaccinia, but smallpox was produced. The disease vaccinia confers immunity not only against vaccinia but against smallpox. The immunity, although not absolute, is stronger than is developed by most other infectious diseases. Vaccinia differs from smallpox in three striking respects:

First. The period of incubation is shorter, being in man 5 days. The incubation period of smallpox is 12 days.

Second. In vaccinia there is no general exanthem. There may be a few vesicles around the point of inoculation, but they develop simultaneously with and not after the main lesion and are probably due to a distribution of the virus at the time of inoculation.

Third. For the development of vaccinia it is necessary that the virus directly reach a susceptible epithelial surface. It may be placed on such a surface or it may be carried there by the blood after having been injected into the blood circulation. The disease may also be transferred from individual to individual by immediate or intermediate contact, but there is no evidence that the virus can be transported by the air as can that of smallpox.

It agrees with smallpox in the similarity of the lesion produced by inoculation to the pock and in the fact that both diseases may be produced by the virus of variola.

If material from a smallpox lesion be placed in contact with a susceptible epithelial surface of man or of the monkey, there develops at the site of inoculation a lesion larger, but having the general characteristics of the pock, together with constitutional disturbances and an exanthem less abundant but otherwise similar to the exanthem of smallpox. Immunity to both vaccinia and smallpox follows the disease. All that we know of *variola inoculata* in man is from the old literature. Inoculation of smallpox to confer immunity is no longer practiced in civilized lands. Plehn mentions that it is still practiced among the natives in Central Africa. We know that the disease so produced is incomparably milder than smallpox. The best results were obtained when the inoculation was made superficially. The period of incubation is 8 days. There is no doubt that the practice of inoculating smallpox to confer immunity would have been extensively used and possibly still used were it not for the fact that the inoculated individual is capable of transmitting to others the true disease. There is no qualitative difference in the virus of *variola inoculata* as compared with that of *variola vera*. The disease differs from *variola vera* in its milder course and shorter period of incubation. There are no records of inoculation being made elsewhere than on the skin. The lymph nodes become swollen, but there have been no histological examinations of the skin lesions nor of the internal organs of man.

We believe that the disease which is produced in monkeys by inoculation with smallpox virus most closely corresponds with *variola inoculata* in man and we have so spoken of it. In *variola vera* the infection is due to a virus which can be carried by the air, and infection usually takes place without either mediate or intermediate contact. The monkey is not susceptible to an air borne-virus. The disease was never transmitted from an infected animal to others in the same cage. The monkeys were exposed to the disease in the wards and infected material was placed in the cages with them. Thinking that the anthropoid apes might prove more susceptible, Orang utans were procured from Java and subjected to the same conditions. One of the Orang utans was given a blanket from a smallpox patient, which it used to cover itself with, but without infection ensuing.

The leucocyte reaction in smallpox is striking. In the early skin lesions there is an absence of leucocytes, the blood shows a hypo-leucocytosis, and marrow and spleen show absence of formation of polynuclear leucocytes. There is increased activity in both marrow, lymph nodes, and spleen, but the differentiation of young cells into polynuclear ones does not take place. In the monkey there is none of this. Both the area of inoculation and the exanthem shows an abundance of polynuclear leucocytes, and the marrow shows a leucoblastic activity. It would be most important to know if this were also true of *variola inoculata* in man.

The shortness of the period of incubation in monkeys also speaks in favor of *variola inoculata*, but there is no *variola vera* with which to compare it. The incubation period of vaccinia in man varies as compared with that of animals. The first thought which arises in endeavoring to form an hypothesis in explanation of the difference between *variola vera* and *variola inoculata* in man is that the inoculation is made into a relatively resistant tissue, and before the organisms have time to develop sufficiently and so to infect the blood that an extensive skin eruption is produced by embolism, the organisms are destroyed or rendered inert by the immune substance. This would satisfactorily explain the mild course but not the short incubation period. The incubation period in the monkeys was found to be very definite. It did not materially vary whether the inoculation was made in the trachea, or by blowing dried virus into the lungs, or by injecting it into the blood. In the inoculated monkeys the lesions in the bone marrow and testicle, which we have learned to regard as a characteristic feature of smallpox, are absent. In one monkey only, in which an abundant exanthem followed intratracheal inoculation, a single characteristic lesion was found in a seminal vesicle. We do not know that a virus similar to that which produces the infection in *variola vera* is formed in the monkey. The only way this could be proven would be by exposure of nonprotected individuals.

Variola inoculata in the monkey differs from *variola vera* in the relatively smaller numbers of the intranuclear parasites which are present. The same forms are found as in man, but are so few that a prolonged search may be necessary to find them. They were found in greatest numbers in 2 cases, one an Orang utan and the other a Philippine monkey inoculated in the trachea. Of course, we know nothing as to the relative abundance of intranuclear forms in *variola inoculata* in man.

Certain experiments were made in the Philippines with reference to the immunity produced by vaccinia as compared with that produced by *variola inoculata*. These experiments were not sufficiently numerous and varied to cover the entire field. However, they show certain interesting features. The immunity produced by vaccinia is stronger and more fully protective than that produced by *variola inoculata*. Further, vaccinia is a more potent virus than that of variola. It was found easier to produce immunity to *variola inoculata* than to vaccinia. The evidence is that

the immunity is germicidal in character for the serum of an immune animal renders vaccine virus inactive. We have not been able to make any tests with the serum of monkeys immunized by the variola virus. Experiments made to test the influence of unfavorable external conditions on the virus of vaccinia and variola showed that vaccinia was much more resistant. The variola virus seems to undergo an attenuation after passing through a series of monkeys, finally losing the power of producing an exanthem, although a typical local lesion followed inoculation. The same was true of variola virus which had been subjected to the influence of glycerin for various periods.

It is generally believed that in man the primary variola infection takes place on some mucous surface, systemic infection following from the development of the organisms at the primary focus. There is no anatomical evidence for this assumption. Such a protopustule has never been found. In the 52 autopsies made in Boston careful search was made for such a lesion, but in vain. The period of inoculation in smallpox runs its course without symptoms, but it would be possible for such a lesion to exist in the lungs without producing symptoms, as can a considerable tuberculous focus. We have rarely produced the evidence of systemic infection shown either by immunity or exanthem by inoculating on the mucous membrane of the nose, mouth, or palate, or on the cornea. In these places, owing to the absence of a dense, horny layer which would retain the products in the lesion, an open ulcer was formed and absorption was prevented. Systemic infection did result both from intratracheal inoculation and from blowing the dried virus into the lungs. In this case absorption would take place both from the lesion and from the mucous surface. The type of disease produced in the monkey was not affected by varying the place of inoculation. By insufflating the virus into the lungs a peculiar form of pneumonia was produced with proliferation of the alveolar epithelium and with the cytoplasmic forms of cytoryctes in the epithelial cells. The evidence shows that variola infection can take place in the lungs.

The work in Manila has confirmed what was stated in the earlier publications concerning the cytoryctes, but has added nothing. We feel sure from our work that the inclusions in the cells of the lesions are living organisms. It seems also sure that the organism described does not conform to the type of the other known organisms. The evidence that the things described are living, comes in part from the analogy of structure with other things which are generally recognized as living organisms, and in part from the analogy with living things which they give by progressive growth and differentiation of structure. Certain forms are found at certain intervals of time and they occur in sequence. It has not been possible to show in them nuclear material with the Romanowsky stain nor, so far as I know, has it been possible to stain with this the nuclei of malarial parasites in tissues. In the investigation of smallpox we are

unfortunately limited to the tissues. We have never been able with certainty to detect either in the virus of smallpox or vaccinia, or in the blood of an infected animal or man, the forms which in the tissue we recognize as parasites. If the very minute bodies which we speak of as gemmules in the cytoplasmic cycle and as spores in the nuclear cycle were present in such fluids, we do not know how they could be recognized. The bodies can only be regarded as parasites or as products of cell degeneration. If degenerations, they are totally unlike any of the ordinary substances found in degenerating cells. Moreover, their presence inaugurates the cell changes which are found in the lesions. The cytoryctes occur in cells which but for their presence show no departure from the normal type. They are specific. No other disease shows the same changes in the cells. If degenerations, it would be necessary to assume that the virus of smallpox causes certain cells to produce substances within them which have a certain form and size and which grow and change their structure with growth. In other diseases such as *molluscum contagiosum* and in the *epithelioma contagiosum* of fowls we find substances in the cells which are regarded as degenerations and which in their mass and extent are specific. But the character of the degeneration is not specific, and the same changes are found in single cells in other processes. Along with the inclusions in cells, which are definite in size and form and which we recognize as parasites, there are others which are irregular and indefinite. We are inclined to regard these in part as imperfectly developed or degenerated parasites. The immune substance produced in variola and vaccinia has been shown to act as a germicide. It may be formed in the lesion or elsewhere, but it certainly is present and may exert its influence on the organisms which are present. We believe that the inclusions are living organisms for the reason given and that they are the cause of the disease because their relation to the lesions is that of other causal organisms.

In our work on smallpox which is now nearing its temporary conclusions, certain questions have presented themselves. These questions relate: First, to the parasite and its life history. Second, to the interrelationship of *vaccinia*, *variola inoculata*, and *variola vera*. Third, the immunity and its mode of production. Fourth, to the mode of production of the exanthem. Fifth, to the mode of infection in *variola vera*. In our work both in Boston and Manila we feel that we have made some contributions to all these questions. None of them are completely answered. Their answers involve long and arduous work by skilled investigators, on both vaccinia, variola in man, and the experimental disease in monkeys. For the work a constant supply of virus and of animals for experiment is necessary, and we believe that the work can only be carried out in places where both these conditions can be fulfilled. The most important contribution which could at present be made would be the discovery of an animal in which *variola vera* can be experimentally produced.

A CRITICAL REVIEW OF THE LITERATURE ON EXPERIMENTAL VARIOLA AND VACCINIA IN THE MONKEY.

The literature bearing upon the reactions of the monkey to inoculation with the virus of vaccinia or of variola is to be found in fifteen publications, which are briefly summarized below. These articles will first be considered separately in chronological order and the findings will then be combined so as to present a connected account of our present knowledge of the subject:

Zulzer, 1874, attempted to produce variola in "Cercopithecus." This author describes briefly the results of experiments on 5 monkeys. Two animals were fed with a mixture of bread and variolous material. No disease followed this procedure. The hair was clipped from an area on the back of one monkey, care being taken not to injure the skin, and variola virus was put in contact with the skin, where it was allowed to remain for three hours. The animal showed no reaction. One monkey was inoculated on the skin with blood from a case of hæmorrhagic variola. This monkey showed a rise in the body temperature which ranged between 40.8° and 41.4° C. from the sixth to the eleventh day of the experiment. A profuse general exanthem developed. One monkey was given dried variola virus to play with and in this animal the same phenomena was seen as in the animal inoculated with the variolous blood.

Buist, 1887, records certain experiments which are only of value in that they showed the susceptibility of the monkey to variola and to vaccinia.

Copeman, 1894, reports a rather extensive series of inoculations of "Rhœsus" monkeys with variola virus and with 2 sorts of vaccine virus. He found them susceptible to all 3 viruses and also found that each virus protected against a second inoculation with the others. Two monkeys inoculated subcutaneously with vaccine virus were later shown to be immune to skin inoculation with the same virus. One monkey received an intraperitoneal injection of oxalated plasma from a monkey which had been rendered immune to vaccinia and to variola by inoculation. This animal was vaccinated 14 days later and it was noted that the lesions did not develop as well as upon a control monkey inoculated at the same time with the same vaccine virus. This author finds that the acme of the lesion at the site of inoculation is on the eighth day, both when vaccine and when variola virus is used. Vesiculation is not so marked in the pock resulting from variolation as in that following vaccination. A general exanthem was noted in some of the monkeys after inoculation with variola virus. These monkeys also showed constitutional reaction to the inoculation, as was evidenced by fever, diarrhœa, suffusion of the eyes, and some malaise. The temperature reaction was more marked in them than in the monkeys inoculated with vaccine virus.

In 1895 Sternberg reported the results of experiments by Reed in immunizing monkeys against vaccine with the serum of vaccinated calves and monkeys. *Cercopithecus mona*, "*Rhœsus*," and *Cobus apella* were employed. In two instances

C. mona was shown to react in a typical manner to skin inoculation with vaccine virus. "Rhœsus" also yielded a typical reaction, but in *C. apella* the lesion following vaccination pursued a milder course. The author found that the serum of a vaccinated monkey protected *C. apella* against subsequent vaccination. The serum of the vaccinated calf, even when given in large amounts, only retarded vaccination.

De Haan, 1896, reported upon an extensive series of inoculations of *Macacus cynomologus* with vaccine, retrovaccine, and variola virus. Forty-three monkeys were used in this research. This author found *M. cynomologus* to be susceptible to all 3 of the viruses used. Only a local lesion followed inoculation with vaccine, while a general exanthem was observed in 25 per cent of the monkeys inoculated with variola virus. Both retrovaccine and vaccine protected this species of monkey against subsequent inoculation with variola virus. The acme of the vaccine lesion in this monkey was reached on the seventh day, as in man, while in the calf the lesion was at its height on the fifth day. Vaccine, retrovaccine, and "la variole mitigée" tended to die out if transferred too long on one species. A strain of vaccine virus which became attenuated was reactivated by transferring it to another species and then inoculating it back on the original species. Vaccination of the skin of the monkey protected the animal against subsequent inoculation of the skin with vaccine virus. A strain of vaccine virus gave typical lesions for seven passages through the monkey. The series of animals so inoculated showed the same immunity to subsequent inoculation with vaccine virus. Monkeys successfully inoculated on the skin with vaccine virus were immune to subsequent inoculation of the skin with variola virus. A strain of variola virus was carried from one monkey to another for seven passages, in each case yielding good primary lesions but no general exanthem. After 6 or 7 such passages the strain was inoculated on the calf and produced a vaccine-like lesion. The lesion rendered the calf immune to subsequent inoculation with vaccine virus.

Reed, 1897, found peculiar bodies in the blood of vaccinated and variolated monkeys, but the various controls showed similar bodies and he was not able to produce evidence either of their specificity or of their parasitic nature.

Béclère, Chambon, and Menard, 1899, inoculated three "Macacus" monkeys with variola virus. Primary lesions developed at the site of inoculation which were identical with those following inoculation with vaccine virus. The monkeys showed a constitutional reaction evidenced by diarrhœa, fever, œdema, and albuminuria, and died on the fourteenth day. The serum of such a monkey was shown to have antivirulent properties when put in contact with vaccine virus.

Roger and Weil, 1902, produced lesions by inoculating "Macacus" monkeys with variolous material. Two animals which were inoculated on the skin with variola virus developed typical lesions at the site of inoculation. These monkeys were subsequently shown to have acquired immunity to a second inoculation of the skin with variola virus. One monkey was inoculated on the skin with blood from a case of hæmorrhagic variola. A few small pustules developed at the site of inoculation. Four monkeys inoculated subsequently with blood from a case of hæmorrhagic variola showed no specific lesions. One of these animals died of septicæmia. The monkeys inoculated on the skin with variola virus and the one inoculated on the skin with variolous blood yielded a positive, though imperfect, reaction, when subsequently inoculated on the skin with vaccine virus. Two of the monkeys which had been inoculated subcutaneously with variolous blood were immune to skin inoculation with vaccine, while in one an abortive lesion resulted.

Ewing, 1902, inoculated a "Rhœsus" and a "large African monkey" with variola virus. Both animals gave a positive reaction to the inoculation. He also inoculated monkeys with bacteria of various sorts which had been isolated from cases of variola, always with negative results.

Park, 1902, inoculated 6 monkeys on the skin with variola virus. All the animals yielded a typical pock at the site of inoculation. Both fresh and dried virus was found active on the monkey. He found monkeys refractory to inoculation with virus from cases of varicella and points out the value of such a test in diagnosis of obscure cases where a differential diagnosis must be made between variola and varicella. This author considers "Java" monkeys most suitable for the test.

Blaxall and Fremlin, 1903, show that by feeding monkeys with vaccine virus mixed with their food, specific lesions may result in the mouth (one case), or the mesenteric lymph nodes (one case) may become a locus of the virus. Their results were controlled by inoculations on the calf.

Magrath and Brinckerhoff, 1904, found "Macacus" and "Rhœsus" monkeys to be susceptible to inoculation with variola virus. They call attention to the fact that the disease produced is related to *variola inoculata* in man rather than to *variola vera* and that the specific lesions produced in these monkeys contain *Cytoryctes variolæ*. In a second paper the minute study of the specific lesions is presented and it is shown that both the cytoplasmic and the nuclear forms of the parasite are present.

Magrath and Brinckerhoff, 1903, in studying the blood of variolated and normal monkeys found a variety of bodies which they interpreted as derivatives of the blood cells of the animal.

The difficulty of getting monkeys in those places where the bulk of scientific work is carried on explains the scanty data to be found in the literature upon the reactions of this animal in variola and vaccinia. As the foregoing summary shows, the only worker who had animals enough to do adequate controls and to inoculate a sufficient number for the solution of his problems was De Haan, who worked in Java, where monkeys were abundant. In spite of the comparatively small number of experiments recorded by other investigators, it seems worth while to combine the data in the following summary.

That the monkey is susceptible to vaccinia or to variola has been the experience of all students of the subject. In some cases the species of monkey used is known, and we find that *Macacus cynomologus* is susceptible to variola and to vaccinia (De Haan), *Cercopithecus mona* and *Cebus apella* are susceptible to vaccinia (Reed). "Rhœsus" monkeys were successfully inoculated with vaccine virus by Copeman and by Reed, and with variola virus by Copeman, by Ewing, and by Magrath and Brinckerhoff.

"Macacus" monkeys were found susceptible to variola virus by Béclère, Chambon, and Menard, by Roger and Weil, and by Magrath and Brinckerhoff. An "African" monkey (Ewing), "Java" monkeys (Park), and a "Cercopithecus" (Zulzer) have also been shown to react to inoculation with variola virus. It is unfortunate that the species of monkey used by various investigators is not known, as it may be possible, from statements made in the literature, that different species of monkeys may present different degrees of susceptibility both to the contagium of variola and to that of vaccinia.

The acme of the process at the site of inoculation in vaccination is placed by Copeman at the eighth day of the disease. De Haan finds it

at its height on the seventh day. Copeman notes that the vesiculation is more marked in vaccination than in variolation. Reed finds that *Cebus apella* presents vaccine lesions which run a milder course than they do in the other monkeys used by him. Several investigators compare the lesion produced in the monkey by inoculation of the skin with vaccine virus, with that which develops in the calf and speak of them as being identical.

The constitutional reaction of the monkey to vaccination is mentioned by Copeman and said to be less intense than that following variolation, at least so far as is evidenced by the body temperature.

A general exanthem has never been observed to follow inoculation of the skin of the monkey with vaccine virus.

The immunity reactions of the monkey after vaccination have received considerable attention. Copeman reports that vaccination of the skin of the "Rhœsus" monkey protects against subsequent inoculation of the skin with either vaccine or variola virus. De Haan found the same protection to be conferred upon *Macacus cynomologus* by vaccination.

The protective power of the serum of a vaccinated animal has been tested by several workers. Copeman reports that the oxalated plasma of a monkey, immune to vaccination and variolation of the skin through inoculation, has a slightly modifying effect upon the course of a vaccination in a monkey into whose peritoneal cavity the plasma has been introduced. He also finds that the monkey is rendered immune to vaccination by the subcutaneous inoculation of vaccine virus. Reed finds that the serum of a vaccinated monkey protects *Cebus apella* against subsequent skin inoculation with vaccine virus. The serum of vaccinated calves was not so efficacious in this respect.

Certain miscellaneous experiments are of interest. De Haan finds that vaccine virus tends to die out if transferred too often on one species of animal. The fact that he worked in the Tropics may have influenced his results. Blaxall and Fremlin show that vaccine virus in the food of a monkey may produce lesions in the mouth or may enter the mesenteric lymph nodes and be demonstrable there by inoculation of an emulsion of the nodes upon the skin of the calf.

Inoculations of the monkey with variola virus have been undertaken by a number of observers. In all cases the monkey has been shown to be susceptible to the contagium.

The contents of the specific skin lesion of variola in man has been used for inoculation by Zulzer, Copeman, De Haan, Béclère, Chambon, and Menard, Roger and Weil, Ewing, Park, and by Magrath and Brinckerhoff. As a rule the virus was used in the fresh state, but Zulzer and Park have both employed dried virus as well. The blood from a case of hæmorrhagic variola was used by Zulzer and by Roger and Weil.

Inoculation of a scratch or scarification on the skin has been used as the mode of introducing the contagium in a majority of the experiments. Simple contact with the unbroken skin as well as exposure to dried and

finely divided virus was tried by Zulzer. The subcutaneous injection of blood from a case of hæmorrhagic variola was done by Roger and Weil.

The data on the constitutional reaction of the inoculated animal are rather scanty. Fever has been noted by Zulzer, by Copeman, by Béclère, Chambon, and Menard, and by Magrath and Brinckerhoff. Diarrhœa was observed by Copeman and by Béclère, Chambon, and Menard. Three investigators report animals dying after inoculation of the skin with variola virus. In the case of Béclère's animals it seems evident that there was intercurrent disease. The animals inoculated with fatal results by Roger and Weil and by Magrath and Brinckerhoff were shown to have died either from streptococcus septicæmia or from tuberculosis.

In all experiments where the skin was inoculated, a pock which closely simulated that produced by vaccination was observed to develop at the site of inoculation.

A general exanthem was observed by Zulzer, Copeman, De Haan, and by Magrath and Brinckerhoff. The virus seemed to have lost this power of producing an exanthem when transferred from one monkey to another (De Haan).

The immunity developed by inoculation with variola virus has been studied by Copeman and by Roger and Weil. The former found that variolation of the monkey protected against subsequent inoculation with vaccine or variola virus. The latter found that inoculation of the skin with variola virus did not confer complete immunity to later inoculation with vaccine, although the animals were immune to variolation by skin inoculation.

That the serum of a variolated monkey had "anti-virulent" properties when put in contact with vaccine was shown by Béclère, Chambon, and Menard.

It was shown by De Haan that a strain of variola virus which had been passed from one monkey to another for six or seven generations was *inoculable* on the calf.

A careful analysis of the evidence in the literature seems to show that the disease produced in monkeys by various inoculations with variola virus has always been one which conforms closely to the type of *variola inoculata* as seen in the human subject. This was emphasized by Magrath and Brinckerhoff.

We find no reference to the occurrence of smallpox on monkeys in the wild state among the species inhabiting the Old World. The statement is sometimes made that monkeys in the Western Hemisphere suffer from epidemics of the disease in localities where smallpox is epidemic among men. This statement seems to be based upon the following data.

Andrew Anderson, in his book on Fevers, gives an excerpt from a letter that he received from a friend who was traveling in Central America. This statement has frequently been referred to and it seems worth while here to give it in full.

In the year 1841 I was in the Province of Veragua, in New Granada, to the north of the Isthmus of Panama and left the town of St. Jago on the western coast for David in Chiriqui, a town in the interior, about 60 or 70 miles to the northeast (and leeward) of St. Jago. The smallpox was raging with great violence in St. Jago, but there was no appearance of it in David. A few days after my arrival there, taking my customary morning's ride in the forest, which teems with animal life, I was struck by observing one or two sick and apparently dying monkeys on the ground under the trees. The next morning I was struck by the same singular appearance (for it is very unusual to find a wild animal sick—they instinctively hide themselves) and by thinking that I perceived several on the trees, moping and moving about in a sickly manner, I consequently dismounted and carefully examined two, which were on the ground—one dead and the other apparently dying; and after careful examination, no doubt remained in my mind that they were suffering and had died from smallpox. They presented every evidence of the disease, the pustules were perfectly formed, and in one instance (that of the dying one) the animal was nearly quite blind from the effects. A few days afterwards (I think about four or five days) the first case of smallpox appeared amongst the inhabitants of David, and in the course of a fortnight one-half of the population was stricken.

In 1858 Dr. Furlong, taking part in a discussion upon diseases in animals, stated that he had received a letter from the wife of a prominent physician of the Island of Trinidad who mentioned that during epidemics of smallpox in that island the wild monkeys suffered from the disease.

Charles Kingsley makes the same statement with regard to an epidemic of smallpox which visited the same island in 1739. We have not been able to find where this author got the information.

In view of the statements cited above one can not deny that the monkey may contract smallpox from man. It is probable that different species of monkeys show different degrees of susceptibility to the contagium. The New World monkeys differ in many respects from their relatives in the Old World and it is quite possible that they are more susceptible to smallpox. The monkeys which are generally used in experimental work in smallpox come from the Eastern Hemisphere. We believe that notable increase in our knowledge of smallpox waits on the finding of an experimental animal in which *variola vera* can be produced. The susceptibility of the New World monkeys in this respect should be tested.

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PART I.

STUDIES UPON EXPERIMENTAL VACCINIA IN THE PHILIPPINE MONKEY (MACACUS CYNOMOLOGUS).

I. VACCINIA FOLLOWING INOCULATION OF THE SKIN OF THE MONKEY.

Technique.—Different strains of vaccine virus were employed. For convenience the different viruses will be indicated by Arabic numerals The source of each strain of virus is as follows.

Virus 1.—Supplied by the Serum Laboratory of the Bureau of Government Laboratories,[4] Manila. A description of the method of its preparation will be found in the Third Annual Report of the Superintendent of Government Laboratories.

Virus 148.—From the New York Board of Health. The pulp was mixed with 60 per cent glycerin.

Virus 246.—From the Japanese Imperial Board of Health. The pulp was mixed with 60 per cent glycerin which contained 1 per cent carbolic acid.

Virus 236.—From the Vaccine Laboratory of Park, Davis & Co. The pulp was mixed with 60 per cent glycerin.

Virus 251.—From the Laboratory of Chambon of Paris. The pulp was mixed with 60 per cent glycerin containing 1.5 per cent carbolic acid.

We are indebted to Dr. J. J. Kinyoun, of Glenolden, Pennsylvania, for the last 4 of these viruses. He sent us a quantity of each of these strains and so arranged matters that, through the courtesy of the steamship company and of the United States Marine-Hospital Service, the virus was kept on ice from the time it left his hands until it reached us in Manila.[5]

The skin of the abdomen was chosen as the site of inoculation. The hair was first shaved from a large area and the skin then scrubbed with soap and water, followed by alcohol. We have found the most satisfactory results to follow the inoculation of a shallow incision. As a rule a number of such scratches were inoculated in each animal. When inoculation is done in this way, the study of the development of the lesion can be made to greater advantage than when scarification is practiced; for the lesion produced by the inoculation instrument is so slight that it heals

[4] Now Bureau of Science.

[5] We take this opportunity of expressing our gratitude to Dr. Kinyoun for his kindness in sending us these strains of virus and to those who assisted in forwarding them.

before the specific process, due to the introduction of the virus, has become visible to the naked eye. The vaccine lesion therefore develops in a skin practically normal and its evolution can better be studied than when the phenomena of repair of a scarification complicates the picture. A general anesthetic was employed whenever the operation might cause discomfort to the animal.

Daily observations were recorded upon the constitutional reaction of the animal, the temperature reaction per rectum, the reaction of the lymph nodes, and the macroscopic appearances of the specific lesion.

The material for histological study of the process in the skin was collected by excision of lesions. In many instances the animals were killed at various times after the inoculation. In these cases a complete autopsy was performed and the material saved both from the specific lesions and from the viscera. All tissues were put in Zenker's Fluid for 24 hours, then washed in running water for 24 hours, and hardened by passage through graded alcohols. Material for histological study was embedded by the chloroform-paraffin method. Sections were stained in various ways.

EXPERIMENTS IN DETAIL.

The experiments on which this article is based comprise the inoculation with vaccine virus of 28 monkeys on the skin of the abdomen. The following experiments are selected to be given in detail.

I. *Clinical course of the disease.*

No. 91. Adult male *M. cynomologus.* Inoculated in 12 places on the abdomen with vaccine virus No. 251. Body temperature 38°.5 C.

Twenty-four hours after inoculation 2 scratches show slight elevation, others show no reaction. Body temperature 38°.2 C.

Forty-eight hours. There is slight elevation and some opacity along the line of inoculation. The axillary lymph nodes are of normal size. Body temperature 39° C.

Three days. There is a distinct elevation with opacity and redness about the narrow crust which marks the line of inoculation. The axillary lymph nodes are slightly enlarged. The body temperature is 39° C.

Four days. A distinct pink elevation is present, extending from 2 to 3 millimeters on either side of the central crust. One lesion appears to be vesicular. Axillary lymph nodes distinctly enlarged. Body temperature 39°.5 C.

Five days. The lesions present as rounded elevations from 4 to 5 millimeters across. The central crust is surrounded by a narrow translucent vesicle which in turn is bordered by a pink areola. Axillary lymph nodes markedly enlarged. Body temperature 40°.2 C.

Six days. The lesions show the same features; but the extent is greater and the whole lesion more sharply circumscribed. Axillary lymph nodes enlarged. Body temperature 39°.5 C.

Seven days. The lesions present a central yellowish crust which is bordered by a vesicle, translucent peripherally but opaque about the crust. The lesions are surrounded by a distinct red areola. Axillary lymph nodes enlarged and hard. Body temperature 39°.5 C.

Eight days. The lesions show a slight increase in the extent of the central crust. The vesicular zone has become entirely opaque in places. The axillary lymp nodes are much enlarged. On the right side they form a mass 2 centimeters in diameter. Body temperature 40° C.

Nine days. The lesions present as flat-topped elevations consisting of a peripheral vesicular ring which has become completely opaque, surrounding a macerated area where the crust has been picked off. The whole lesion is surrounded by a pink flush. Axillary lymph nodes enlarged but not so hard as before. Body temperature 39°.4 C.

Ten days. The lesions show an elevated pink, somewhat ragged epithelial edge which surrounds an area which is in part crusted and in part ulcer-like. Axillary lymph nodes somewhat enlarged. Body temperature 39° C.

From this time on the lesions simply showed the phenomena of repair and healed after a few days.

Twenty-three days after the first vaccination the animal was again inoculated on the skin of the abdomen with vaccine virus No. 148. No reaction followed this inoculation.

No. 96. Adult male *Macacus cynomologus.* Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 236. Body temperature 39°.6 C.

Twenty-four hours after inoculation the scratches show considerable elevation with some reddening of the surrounding skin but with no opacity. Body temperature 39°.2 C.

Forty-eight hours. There is slight elevation and some opacity along the line of inoculation. Axillary lymph nodes slightly enlarged. Body temperature 38°.5 C.

Three days. There is an irregular elevation with opacity beside the narrow crust which marks the line of inoculation. Axillary lymph nodes are distinctly enlarged. Body temperature 39° C.

Four days. The elevation which borders the crust has increased somewhat, extending from 2 to 3 millimeters on each side. Axillary lymph nodes much enlarged. Body temperature 39° C.

Five days. Around the crust is a narrow vesicular ring which is bordered peripherally by elevated and reddened epithelium. The lesion is now from 6 to 8 millimeters wide. Axillary lymph nodes large and hard. Body temperature 39°.8 C.

Six days. The lesion presents as a sharply circumscribed elevation consisting of a depressed central yellow crust bordered by an elevated ring from 2 to 3 millimeters broad which is opaque and vesicular near the crust and which is pink externally. Axillary lymph nodes enlarged, some measuring 1 centimeter in diameter. Body temperature 39°.2 C.

Seven days. The lesion is the same as yesterday save that the crust has extended and encroached upon the vesicular ring. Axillary lymph nodes enlarged and hard. Body temperature 38°.8 C.

Eight days. The animal has picked off the crusts and the lesion presents a central excoriated area surrounded by the more or less ruptured vesicular ring. There is a distinct reddening of the skin about the lesion. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Nine days. The crust has formed again in the center of the lesion and about this is to be made out the remnants of the vesicular ring. The areola persists. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Ten days. From this time on the lesions have lost their specific character and simply present the phenomena of healing. The axillary lymph nodes show a diminishing tumor and consistency but are harder than normal for a considerable time after the healing of the skin lesion.

Twenty-three days after the original vaccination the animal was again inoculated on the skin of the abdomen with vaccine virus No. 148. No reaction followed this second inoculation.

No. 98. Adult male *Macacus cynomologus.* Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 1, Lot No. 365. Body temperature 39°.4 C.

Twenty-four hours after inoculation the scratches show slight elevation and opacity. Axillary lymph nodes normal. Body temperature 39° C.

Forty-eight hours. About the inoculation the skin is slightly elevated and opaque. Axillary lymph nodes normal. Body temperature 39° C.

Three days. There is some reddening and marked elevation and opacity of the skin which borders the narrow crust which marks the line of inoculation. Axillary lymph nodes distinctly enlarged. Body temperature 39° C.

Four days. The central crust has increased in extent and is surrounded by a pink elevation from 2 to 3 millimeters broad. Axillary lymph nodes enlarged. Body temperature 38°.4 C.

Five days. The skin immediately about the central crust is slightly translucent, suggesting beginning vesicle formation. Axillary lymph nodes enlarged and hard. Body temperature 39°.5 C.

Six days. The lesion presents a depressed yellow-brown crust which is surrounded by an elevated ring which is sharply circumscribed and is translucent near the crust. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Seven days. The vesicular ring about the central crust is now more or less opaque. There is distinct areola. Axillary lymph nodes enlarged and hard. Body temperature 39°.8 C.

Eight days. Lesions present same features as yesterday save that there is some induration of the subcutaneous tissue beneath the lesions. Axillary lymph nodes very much enlarged. Body temperature 40°.2 C.

Nine days. The central crust has begun to encroach upon the vesicular ring and the areola is less marked. Some degree of subcutaneous œdema persists. Axillary lymph nodes enlarged and hard. Body temperature 40° C.

Ten days. The lesions are still active but only remnants of the vesicle can be made out about the spreading crust. From this time on the lesions lose their specific characters. The enlargement and induration of the axillary lymph nodes suffer a continuous diminution, although the consistency is greater than normally for some days after the healing of the specific lesion is complete.

Twenty-three days after the first inoculation the animal was inoculated again on the skin of the abdomen with vaccine virus No. 148, but no reaction followed.

No. 101. Adult male *Macacus cynomologus.* Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 246. Body temperature 39°.2 C.

Twenty-four hours after inoculation the scratches show a slight opacity but no elevation. Axillary lymph nodes normal. Body temperature 39°.8 C.

Forty-eight hours. There is now some elevation as well as opacity along the line of inoculation. Axillary lymph nodes normal. Body temperature 37°.8 C.

Three days. The elevation and opacity is more marked and now extends for a distance of 2 or more millimeters on each side of the scratch. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. The lesions present as irregular pink elevations which are surmounted by a narrow linear crust which occupies the site of the inoculation scratch. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Five days. Lesions show a depressed central crust bordered by an elevated zone which is translucent near the crust. The whole lesion measures 8 millimeters across. Axillary lymph nodes enlarged. Body temperature 39° C.

Six days. The central crust has enlarged and the surrounding elevated ring is opaque and vesicular for 2 millimeters about the crust. The areola is bright and the whole lesion sharply circumscribed. Axillary lymph nodes enlarged. Body temperature 39° C.

Seven days. The lesion is the same as yesterday save for an increase in the area of the crust and a filling out of the vesicular ring. Axillary lymph nodes slightly enlarged. Body temperature 40° C.

Eight days. Lesion shows the same features but the whole lesion has ceased to spread. The depression of the central crust is marked. Axillary lymph nodes enlarged and hard. Body temperature 38°.8 C.

Nine days. The crust has been picked off and the lesion presents an excoriated area about which the remnants of the vesicle can be made out. Axillary lymph nodes less enlarged but hard. Body temperature 39°.4 C.

Ten days. The lesions are now healing and have lost their specific characters. Axillary lymph nodes but slightly enlarged. Body temperature 39°.6 C.

The further course of the lesions showed only the healing process. The axillary lymph nodes were of normal size and of only slightly increased consistency. In this case the lymph nodes did not show as much reaction as is usual after skin inoculation with vaccine.

Twenty-three days after the original inoculation the monkey was vaccinated a second time on the skin of the abdomen with vaccine virus No. 148. No reaction followed the second inoculation.

No. 105. Adult male *Macacus cynomolgus*. Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 148. Body temperature 40° C.

Twenty-four hours after inoculation the scratches show no reaction. A narrow crust marks the line of each inoculation. Axillary lymph nodes normal. Body temperature 39°.5 C.

Forty-eight hours. There is a slight elevation which is opaque and a faint pink color about the crust. Axillary lymph nodes normal. Body temperature 38°.8 C.

Three days. About the scratch the epithelium is elevated, opaque, and pink for a distance of 2 or more millimeters. On pressure a small amount of clear serum oozes from under the crust. Axillary lymph nodes slightly enlarged. Body temperature 38° C.

Four days. The lesion presents as a sharply circumscribed, pink elevation 3 millimeters in width which surrounds a slightly depressed crust. Axillary lymph nodes enlarged. Body temperature 39°.8 C.

Five days. The central crust has enlarged and there is a translucent vesicular ring. Axillary lymph nodes enlarged and hard. Body temperature 39°.8 C.

Six days. The depressed yellow central crust is now bordered by an elevated ring in which can be distinguished an opaque, yellowish zone which merges with a translucent band of a gray color. The areola is distinct and bright red. Axillary lymph nodes enlarged. Body temperature 39°.6 C.

Seven days. The lesion has attained a total width of 1 centimeter. Some subcutaneous œdema is apparent. Axillary lymph nodes enlarged and hard. Body temperature 39°.8 C.

Eight days. Lesions show same features as yesterday. Axillary lymph nodes slightly enlarged. Body temperature 40°.1 C.

Nine days. The crusts have been picked from the lesions and have left an excoriated area surrounded by a ragged, opaque, epithelial edge about which the skin is reddened. Axillary lymph nodes enlarged. Body temperature 39° C.

Ten days. From this time on the lesions show simple repair. The axillary lymph nodes were more or less enlarged and hard for some time.

The monkey was inoculated on the skin of the abdomen with vaccine virus No. 148 on the twenty-third day after the first inoculation. This second vaccination was followed by no reaction.

SUMMARY.

(1) *Objective description of the specific skin lesion based upon the appearances in twenty-eight monkeys.*—After 48 hours the lesion appears as a simple scratch with more or less opacity and elevation, not extending over 2 millimeters from the line of inoculation.

After 72 hours the elevation may become less in extent and take on a pink color, or it may remain as before.

After 96 hours the elevation becomes more marked and more sharply circumscribed, and may begin to show translucence about the crust which forms along the line of inoculation.

After 5 days the zone of translucence about the crust is more evident and contains fluid, which forms a vesicular ring about the crust. The contents of this vesicle is a clear fluid. The vesicular ring is bordered externally by a zone of red, which fades peripherally. The whole lesion—*i. e.*, crust, vesicle, and areola—forms a rounded elevation, which flows into the general skin surface without a sharp line of demarcation.

After 6 days the various parts of the lesion remain the same as on the fifth day, but there is an increase in the total width. The vesicle ring spreads peripherally, and coincident with this the crust encroaches upon the inner side of the vesicle ring. The inner part of the vesicle ring may present an opaque appearance at this stage. The crust may be more or less depressed in such a manner that the vesicular ring forms a rampart about it.

After 7 days the conditions remain the same, there being more or less spreading of the whole lesion, accompanied by an increase in the extent of the central crust. At this stage, the outer edge of the vesicular ring may present an abrupt declivity, so that the whole lesion forms a flat-topped plateau. The skin proper is thickened and hard, and a subcutaneous œdema is apparent beneath the lesion.

After 8 days the lateral extension of the lesion has ceased and the central crust has encroached upon the vesicular ring. The subcutaneous œdema noted after 7 days is now more noticeable, often presenting itself as a broad indurated base.

From this time on the healing of the lesion proceeds rapidly. The central crust finally occupies all the space previously held by the vesicular ring, being bordered by the pink epithelium which is growing inward from the normal skin. This newly formed epithelium slowly spreads beneath the crust, and finally the latter falls off, leaving a pink and shining scar.

The lateral excursions of the process outward from the line of inoculation amount to from 6 to 8 millimeters, measuring from the center of the crust to the outer edge of the vesicular ring. After 5 days, when the

lesion is first macroscopically analyzable into zones which correspond with the microscopic findings, the growth of the lesion is seen to be due to a lateral extension of 3 elements. These may be described, from without inward, as—

(1) A zone of hyperæmia.
(2) A vesicular ring, composed of—
  (*a*) A zone of translucence.
  (*b*) A zone of opacity.
(3) A crust.

Up to the end of the eighth day of the development of the lesion these move out from the line of inoculation in the order named, each encroaching upon its outer neighbor toward the end of this period, a little more rapidly than the latter spreads. On the eighth day of the development of the lesion this spreading stops, and the zone of hyperæmia slowly fades, the opaque zone spreads over the remnant of the translucent zone, and it in turn is taken up by the spreading of the crust.

The profile of the lesion presents 3 rather distinct phases, as follows: After 5 days it is more or less hemispherical, or at least an arc of a circle. After about 7 days it is flat-topped, presenting a steep declivity on either side, with perhaps a rampart effect from the depression of the crust, and after 8 days the whole lesion may be raised upon a broad swelling, which elevates with it some of the normal epithelium about it.

(2) *Histology of the vaccine lesion of the skin.*—The histogenesis of the vaccine lesion in the skin of the monkey is similar to that of the vaccine lesion in the skin of other mammals. We find the same changes in the epithelial cells leading to vesicle formation, followed by reparative processes resulting in complete healing of the lesion. The histology of the vaccine lesion has been described so often that it seems unnecessary to repeat it here. We find the development of the lesion to be the same after inoculation with different strains of virus.

In the study of our large series of vaccinations of the monkey we have been struck by the extent of the process in the corium and in the subcutaneous tissue beneath the lesion. We can recognize a general inflammatory and reparative process, and besides a series of phenomena which we regard as due to the activities of the specific organism. The former group of phenomena are shown by cellular and fluid exudative processes and by proliferation of connective tissue cells with the new formation of blood vessels, and are such as would accompany any infected wound of the skin. Besides these we find early in the process a marked swelling and proliferation of the endothelial cells of the capillaries adjacent to the specific process in the epidermis. The endothelial cells of the capillaries and lymphatics in these situations are frequently invaded by the protoplasmic phases of *cytoryctes variolæ*. This reaction in the corium is in evidence from the third day of the disease onward. We interpret this condition as due both to the action of the toxines set free from the specific

lesion in the epidermis and to an invasion of the blood vessels by the organism.

The process in the corium and in the subcutaneous tissue beneath the lesion does not present such definite areas of necrosis as will be described in the variolous lesions. This may be due to the fact that the vaccine lymph was glycerinated, and so was free from pyogenic organisms, while the variola virus was untreated and contained streptococci and other pathogenic bacteria. It is to be noted that beneath the vaccine lesions capillaries invaded by cytoryctes were only found near to the infected epidermis while, as will be shown, in variolation the specific process extends deeply into the subcutaneous tissue.

(3) *Constitutional reaction.*—The general condition of the animal does not seem much disturbed. There may be some anorexia about the sixth day, but it can not be said to be constant or marked.

(4) *The temperature reaction* is not very definite, although there is to be derived from it evidence of a constitutional reaction. The most common reaction is a slight rise on the sixth and ninth days of the disease. This fever rarely exceeds 40° C. The temperature reaction is not nearly so typical as in *variola inoculata.*

(5) *The lymph nodes.*—With the development of a vaccine lesion on the belly of a monkey there is a reaction of the axillary lymph nodes. This is shown by an enlargement, first noticeable on the fourth day (after 3 day's development of the lesion), which becomes more apparent on the succeeding days. On the sixth or seventh day the nodes may be 1 centimeter in diameter and tender. With the regression of the lesion the nodes decrease in size but remain more firm than they are normally.

The histology of the axillary lymph nodes of monkeys vaccinated on the abdomen presents the same picture as that seen in the variolated monkeys. Oedema of the sinuses and the presence of phagocytic endothelial cells, red blood corpuscles, and leucocytes, together with a small amount of fibrin, characterize the process in the nodes taken from animals during the active evolution of the vaccine lesion.

(6) A general exanthem has never occurred in our vaccine monkeys (total 28). In some cases secondary lesions occur about the primary lesion (daughter pocks). These run the same course as the primary lesions. Auto-inoculations are not uncommon, but in all instances which we have observed their occurrence was so obviously due to infection from the initial lesion by acts of the animal that the question of their being of the nature of an exanthem could not be raised.

(7) No lesions were found in the internal organs. The bone marrow and testes were found free from the focal lesions which occur in these organs in *variola vera* in man. *Cytoryctes variolæ* was constantly found associated with the specific process in the skin at the site of inoculation. The question of the occurrence of the organism will be considered more in detail in a separate section of this report.

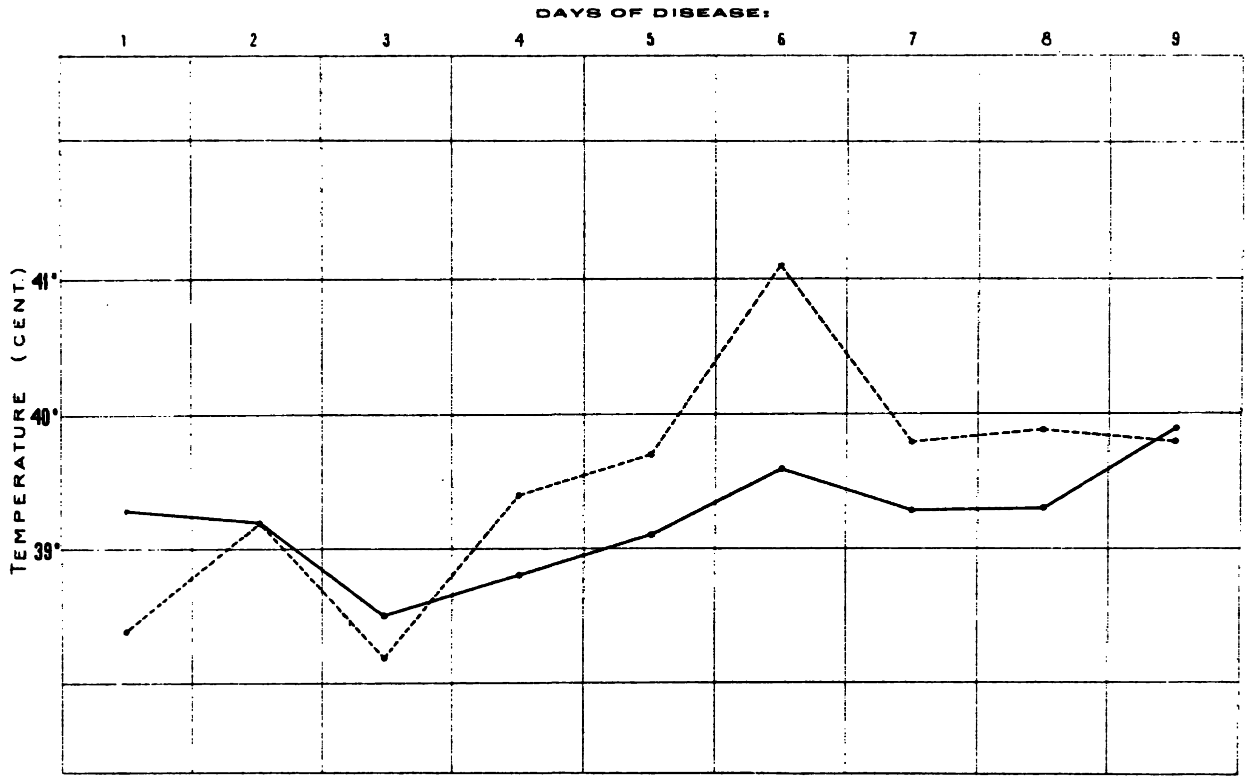
DISCUSSION.

In considering the results of the preceding experiments certain points of considerable theoretical interest present themselves for discussion. Perhaps the most prominent feature of the disease vaccinia is the specific immunity which it produces. At it is hardly possible to consider this immunity without at the same time dealing with variola, we have decided to discuss this phase of the disease in a separate section. There remains for consideration here the clinical feature of the disease vaccinia as it occurs in *Macacus cynomologus*. In our experiments we have found that this species of monkey reacts to an inoculation with vaccine virus on the skin by a definite sequence of phenomena which are reproduced in each experiment with only very slight modifications. It is true that certain animals do not react in exactly the same degree as do others, but these "abortive" reactions seem to us to depend upon conditions of natural immunity and to differences in the virus used for inoculation, and so do not affect the truth of the general statement that this species is markedly susceptible to the disease.

Comparing vaccinia in *M. cynomologus* with vaccinia in man and in the calf we see that we have a strict parallel. We find a lesion developing at the site of inoculation which runs a definite course and heals after a fairly constant interval. This local lesion is associated with enlargement of the lymph nodes. Only those nodes are affected which are interposed between the areas of the skin on which the lesion develops and the main lymph trunks. During the active evolution of the local lesion there is a general reaction of the inoculated animal as shown by a rise in the body temperature. The primary lesion is shown to exist at the site of inoculation from a very early period after the inoculation as a distinct process so that the latent period, before the lesion can be diagnosed by its appearance to the naked eye, is shown to be rather apparent than real.

Two things serve to differentiate the vaccine process from that following inoculation of variola virus on this species of monkey: First, in vaccinia we have never observed an exanthem; second, the temperature reaction is not so definite in its onset as in *variola inoculata*.

FIG. 1. TEMPERATURE REACTION.



VACCINIA AND VARIOLA INOCULATA IN THE MONKEY. (COMPOSITE CURVES.)

Vaccinia ———— Variola inoculata - - - - - - -

The histological peculiarities of the specific lesion of vaccinia in these monkeys are similar in every way to those found in lesions following vaccination of other susceptible animals.

We have seen then that the disease vaccinia in *M. cynomologus* conforms strictly to the type of the disease vaccinia and in a more general way can be included in that group of processes which embraces all forms of variola and vaccinia.

The data furnished by our experiments upon the immunity reactions and upon the differences due to different strains of virus will be presented in another section of this article.

CONCLUSIONS.

(1) Inoculation of the skin of *M. cynomologus* with vaccine virus is followed by the development of a lesion at the site of inoculation which is similar in all respects to that which follows similar inoculations of other animals.

(2) The development of the lesion is associated with a rise in the body temperature which is most marked during its active evolution.

(3) The lymph nodes which are interposed between the area of skin on which the lesion develops and the main lymph trunks, show enlargement coincidently with the temperature reaction. The nodes show histological changes which account for this enlargement.

(4) *Cytoryctes variolæ* is found in the epithelial cells of the vaccine lesion and also in the endothelial cells of capillaries beneath the epithelium of the lesion.

(5) *Macacus cynomologus* is susceptible to vaccinia.

2. VACCINIAL KERATITIS IN THE MONKEY.

Nine monkeys were inoculated and the animals were killed after various periods to obtain material for the histological study of the lesion at different stages of its development.

Technique.—The animal was deeply anæsthetized and the cornea lightly incised with a sharp scalpel. Care was taken not to cut deeply into the corneal substance. A small amount of vaccine was rubbed into the incision. A daily record of the appearances at the site of inoculation and of the general condition of the animal was kept. When the animals were killed a complete autopsy was done, and tissue for histological study was saved from the site of the specific lesion and from the viscera. The animals were under the influence of morphine during the development of the lesion.

EXPERIMENTS IN DETAIL.

No. 13. Monkey inoculated on both corneas with vaccine virus No. 1. After 17 hours the cornea shows no macroscopic evidence of a specific process. Animal chloroformed and tissues preserved for histological study.

Histological examination.—Sections of the cornea show very slight injury to corneal substance, and the defect completely covered over by epithelium. No evidence of any process other than repair.

No. 10. Monkey inoculated on both corneas with vaccine virus No. 1. Twenty-four hours after the inoculation there is a roughness barely perceptible along the line of incision. Animal chloroformed. When the eyes were dropped into Zenker's fluid, minute opaque spots appear at once along the line of inoculation.

Histological examination.—The epithelium has grown over and completely filled the defects in the corneal substance representing the inoculation incisions. Numbers of leucocytes are present in the tissue about the incisions. The epithelium contains many cytoplasmic phases of *cytoryctes variolæ*, which are rather small and occupy cells which are apparently normal.

No. 11. Monkey inoculated on both corneas with vaccine virus No. 1. Twenty-four hours after inoculation there is slight roughness along the line of incision. After 48 hours there is some elevation as well as roughness and a central loss of substance is also apparent. Animal chloroformed and tissues preserved for histological study.

Histological examination.—Section presents two clean cut and moderately deep incisions, one of which is filled with epithelium. The second incision is not filled in and the surface about it is denuded of epithelium for a short distance. Comparatively few polymorphonuclear leucocytes are present about the lesions. The corneal tissue beneath the incisions is œdematous. On one side of the incision, the corneal epithelium is two and a half times thicker than normally. Elsewhere the epithelium is thinner than normal, both near the incisions and for a considerable distance from them. In places, the epithelium is lifted up by an exudate. Cytoryctes are present in the cells of the lesion.

No. 7. Monkey inoculated on the right cornea with vaccine virus No. 1. The left cornea inoculated with fresh virus obtained from a vaccine lesion of the skin of Monkey No. 1; duration 4 days.

Twenty-four hours after inoculation the right cornea shows no macroscopic lesion, while the left cornea is slightly opaque and rough along the incision.

Seventy hours. The right cornea shows slight elevation at the site of inoculation. The left cornea is slightly rough along the line of incision. Animal chloroformed. When the eyes were placed in Zenker's fluid a loss of substance was apparent along the lines of inoculation.

Histological examination.—The corneal surface is denuded of epithelium over an area 0.5 millimeter across, surrounding the site of the inoculation incision. There is at no point any proliferation or thickening of the epithelial layer. It is thin and tapering at the border of the denuded area, and here cytoryctes are present in both the degenerated and the well-preserved epithelial cells. Very few leucocytes are found and when present are usually situated upon the denuded surface of the cornea.

No. 12. Monkey inoculated on both corneas with vaccine virus No. 1.

Forty-eight hours after inoculation there is no appreciable elevation or roughening, but there is distortion of an image reflected on the cornea.

Three days. The left cornea is distinctly rough at the site of the inoculation. The right cornea is very slightly roughened. There is no opacity of the cornea. A small amount of muco-purulent material is present in both conjunctival sacs.

Four days. Condition of corneas the same. Animal killed and tissues preserved for histological study.

Histological examination.—Both corneas present areas about 1 to 1.5 millimeters across, from which the epithelium is lost. In the center of this area the corneal connective tissue presents a minute defect representing the trauma of inoculation. The epithelium tapers to a thin edge about the denuded area and here are found cytoryctes in small numbers. Leucocytes are practically absent from the lesion.

No. 20. Monkey inoculated on both corneas with vaccine virus No. 1.

Twenty-four hours after inoculation both corneas are smooth.

Forty-three hours. Slight distortion of reflected image. No conjunctival discharge and no photophobia.

Three days. A slight depression marks the line of inoculation, about which there is some roughening of the corneal epithelium.

Four days. Marked roughening of cornea about inoculation sites with central loss of substance. Photophobia and some muco-purulent discharge noted.

Six days. Condition same, save that the loss of substance is more marked. Animal chloroformed and tissues preserved for histological study.

Histological examination.—Sections stained with eosin and methylene blue. Vertical sections taken just outside the edge of the area of the cornea denuded of epithelium show a widespread thickening of the epithelial layer and large numbers of cytoryctes at a point nearest the inoculation site.

No. 17. Monkey inoculated on both corneas with vaccine virus No. 1.

Twenty-three hours after inoculation both corneas are smooth, but the line of inoculation can be made out by the distortion of a reflected image.

Fifty-three hours. Both corneas show slight loss of substance, together with some roughening. A slight degree of photophobia is present.

Three days. The corneas are hazy and the loss of substance at the site of inoculation has increased. There is some photophobia and some muco-purulent discharge.

Four days. Marked roughness of the corneal surface is apparent about the inoculation sites. Photophobia and discharge continue.

Five days. Each cornea shows a shallow erosion, about which the cornea is rough and hazy. Photophobia and discharge still present.

Six days. Condition same as yesterday save that the conjunctivæ are œdematous.

Seven days. Macroscopic appearances as before. Animal chloroformed and tissue saved for histological study.

Histological examination.—The condition found is similar to that described in the other corneas of this series. There is an area about the point of inoculation over which the epithelium is wholly deficient. The surface is smooth and the corresponding portion of the cornea is slightly thinner than normal.

The epithelium tapers at the border of the deficiency and at this point contains small numbers of cytoryctes. In one cornea there is a deep incision partially filled with epithelium. The tissue about this contains a small number of leucocytes.

No. 10. Monkey inoculated on both corneas with vaccine virus No. 1.

Twenty-four hours after inoculation the cornea appears smooth.

Forty-eight hours. Corneas show slight distortion of the reflected image.

Three days. Both corneas slightly roughened along the line of inoculation.

Four days. Roughness is more marked and is present over a circumscribed area about the incisions. The cornea appears hazy and a small amount of muco-purulent material is present in the conjunctival sac. Slight degree of photophobia is observed.

Five days. Condition same as yesterday, save that no photophobia is noted.

Six days. A loss of substance is evident at the center of the roughened area. The conjunctival discharge and the photophobia persist.

Seven days. The superficial loss of corneal substance has increased, otherwise the condition remains the same.

Eight days. Animal chloroformed. There is considerable œdema of the lids with a watery discharge. The eroded area has increased in extent. Tissues preserved for histological study.

Histological examination.—The epithelium covering the central portion of the corneal surface is very thin and consists of only two layers of cells. There is deficiency of epithelium over an area not over 0.5 millimeters across. Cytoryctes are present in considerable numbers in a single area of the epithelium.

SUMMARY.

The inoculation of the monkey's cornea with vaccine virus, provided a secondary pyogenic infection does not supervene, results in the production of a lesion which has rather indefinite macroscopic characters. Twenty-four hours after the inoculation a slight roughening, associated with more or less elevation of the corneal epithelium, may be apparent. After 48 hours a loss of epithelium about the point of inoculation is frequently present. The further development of the lesion is associated with an increase in this erosion and with the development of some degree of photophobia and a conjunctival discharge.

The histological characters of the lesion are as follows:

In the corneas taken 17 and 24 hours after vaccination the defect produced by the inoculation trauma is filled in by the growth of the epithelium. This constitutes the ordinary form of repair following simple incision of the cornea. Cytoplasmic forms of Cytoryctes are present in the epithelium. Subsequent to this, up to 8 days and probably for an indefinite period, there is a loss of epithelium over an area surrounding the site of the inoculation. The epithelium is thin and tapers at the edge of the abraded area, and here the cells appear to be undergoing degeneration. Cytoryctes are present in all lesions. Leucocytes apparently play no part in the process but are present in those lesions in which there is injury to corneal connective tissue.

The destruction of epithelium evidently outstrips the growth of the epithelium in the corneal vaccine lesions of the monkey.

DISCUSSION.

When we compare the lesion produced on the cornea of the monkey by inoculation with vaccine virus with that which follows a similar inoculation on the rabbit, we see that, although the fundamental characters of the lesion are the same, there are important differences.

One of the striking features of the vaccine process on the monkey's cornea is the loss of epithelium about the line of inoculation. This loss is apparent in the early as well as in the more advanced stages of the process and probably persists until the active stages are passed. It is evident that the vaccine virus in these experiments produced a more extensive destruction of corneal epithelium than has been observed in the

corneal inoculations of the rabbit, in which the growth of the epithelium over compensates any destructive action. The removal of the degenerated epithelium was probably assisted by the dextrous rubbing of the eyes which was frequently observed with the monkeys inoculated upon the cornea.

The course of development of the vaccinal lesion on the monkey's cornea makes it a less favorable place than we had hoped for the study of the etiological factor of the disease. Owing to the loss of epithelium which takes away the bulk of the infected cells the sections are not rich in parasites. In spite of this, the sections from the corneal lesions yielded important data which will be considered in a later article dealing specifically with *cytoryctes variolæ*.

CONCLUSIONS.

(1) Vaccination of the cornea of the monkey produces a lesion which is specific and which is comparable with that following the same inoculation in the rabbit.

(2) The lesion is characterized chiefly by an early loss of epithelium at the site of inoculation, which is accompanied by the development of photophobia and conjunctivitis.

(3) *Cytoryctes vaccinæ* are present in the cells of the lesion.

3. VACCINIA FOLLOWING INOCULATION OF THE MUCOUS MEMBRANE OF THE MONKEY.

In this section will be presented the results of inoculation of the mucous membrane of the monkey with vaccine virus. The evolution of the lesion at the site of inoculation, the general reaction of the animal, and the histology of the specific lesion will be described in some detail. These inoculations were undertaken to show what variations might result in the evolution of the specific lesion of vaccinia due to a change in the *locus* of inoculation. The material collected for the histological study of the specific lesion also served for the study of the parasite of the disease.

Technique.—Nine monkeys were inoculated in this series. Vaccine virus No. 1 was rubbed into shallow incisions made on the mucous membrane of the soft palate, of the septum of the nose, and of the inner surface of the lower lip. The appearances at the site of inoculation, the body temperature, and the general condition of the animal were noted daily. The monkeys were killed at various intervals after the inoculation, and material for histological study was preserved in Zenker's fluid.

EXPERIMENTS IN DETAIL.

No. 81. Adult male *M. cynomolgus.* Inoculated on the left side of nasal septum, on inside of lower lip, and on the soft palate with vaccine virus No. 1. At the time of inoculation some of the virus was also blown into the throat. Body temperature 39°.9 C.

Twenty-four hours after the inoculation no process was visible at the site of the incisions. Body temperature 38°.5 C.

Forty-eight hours. In the nose the inoculation scratch is just visible. The lip presents a small white spot. The palate shows a minute, white papule 1 millimeter or less in diameter. Body temperature 38° C.

Three days. Nose: Some elevation and opacity of the mucous membrane about a small brown area. Lip: Three circular opaque white areas, 2 millimeters in diameter, along the line of inoculation. Each of these opaque areas shows a minute central pink spot. Palate: In the inoculated area are two small, slightly elevated, opaque white spots, about 2 millimeters in diameter, each with a minute central pink dot and surrounded by an areola. Body temperature 38°.4 C.

Four days. Nose: An opaque white elevation, just inside the orifice. Lip: An elevated opaque white area, 3 × 6 millimeters, the center of which is macerated and which is surrounded by a distinct red flush. Palate: An oval area, 3 × 4 millimeters, opaque-white, with 2 minute translucent dots near the center. The 2 areas noted yesterday have fused. Marked peripheral pink flush. Body temperature 30° C.

Five days. Nose: Left orifice almost closed by swelling of mucous membrane. On the anterior portion of the left side of the septum is an elevated, white area surmounted by a small vesicle surrounding a small yellow crust. Lip: The lesion presents an eroded area with an elevated ragged edge, 10 × 5 millimeters in extent and surrounded by a distinct areola. No trace of vesicle formation can be made out, the lesion being essentially an ulcer. Palate: An elevated opaque white area, 5 × 3 millimeters, with gray, semitranslucent center and surrounded by an aerola. Body temperature 39°.2 C. Animal chloroformed and autopsy done at once. Primary lesions as described. No evidence of a septic lesion found in gastro-intestinal tract. Viscera normal.

No. 82. Adult male *M. cynomologus.* Inoculated on mucous membrane of nose, lip, and palate with vaccine virus No. 1. Body temperature 39°.5 C.

Twenty-four hours after the inoculation the mucous membrane shows no macroscopic lesion. Body temperature 39°.5 C.

Forty-eight hours. A small, white area is visible in the nose and a similar spot is seen at the site of inoculation on the lip. The palate shows no lesion. Body temperature 38°.8 C.

Three days. Nose: A pink elevation is visible. Lip: A white, opaque area, 2 × 5 millimeters, is apparent along the line of inoculation. The mucous membrane over this area is more or less macerated, and about the area there is a distinct red flush. Palate: A single translucent papular elevation, 2 millimeters in diameter, with a minute opaque central dot is seen on the line of inoculation. Body temperature 39°.2 C.

Four days. Nose: A number of minute elevated opaque areas surrounded by a pink flush. Lip: An excoriated, bleeding area, 6 × 3 millimeters, with an elevated, opaque, white edge and surrounded by a distinct areola. Palate: An opaque, white area with an elevated margin and surrounded by a red areola. Body temperature 39°.5 C.

Five days. Nose: A grayish elevation with a pink margin. Lip: An elevated opaque white area, 10 × 6 millimeters, with some erosion of the surface and with a sharp border which is surrounded by a red areola. Palate: An elevated, gray-yellow area, 8 × 3 millimeters, presenting several minute, bleeding points and a bright red areola.

Six days. Nose: Inspection difficult and findings indefinite. Lip: Lesion as before but somewhat increased in extent. Palate: Lesion as yesterday save that it has spread a little. Body temperature 40° C.

Seven days. Nose: Mucous membrane swollen and presents an opaque, white elevation on the septum. Lip: Lesion is 1 centimeter in diameter, border slightly irregular and overhanging in places, central portion depressed and eroded, showing in places red granulation tissue and in places islands of macerated epithelium. The mucous membrane about the lesion is hyperæmic. Palate: An opaque, gray area, 10 × 4 millimeters, edge elevated and surrounded by an areola. Body temperature 39°.5 C.

Eight days. Nose: Yellow crusts are visible, but swelling of the mucous membrane renders close inspection impossible. Lip: Lesion is the same as yesterday save that the areola has faded. Palate: Same as before save that areola is absent and the lesion has not extended. Body temperature 39° C. Animal chloroformed and autopsy done at once. Lesions at site of inoculation as described above; internal organs show no evidence of specific lesions.

No. 83. Adult male *M. cynomologus.* Inoculated on the septum of the nose, inside of the lip and on the soft palate with vaccine virus No. 1. Body temperature 39°.8 C.

Twenty-four hours after the inoculation no specific lesion is macroscopically evident. Body temperature 39°.6 C.

Forty-eight hours. Nose: A narrow red crust is evident. Lip: Palate shows no reaction. Body temperature 38°.8 C. Animal chloroformed and autopsy done at once. Extensive lesions of tuberculosis were present. This case will be described from the point of view of the intercurrent disease in another article.

No. 84. Adult male *M. cynomologus.* Inoculated in the nose, on the lip and palate with vaccine virus No. 1. Body temperature 39°.5 C.

Twenty-four hours after the inoculation no evidence of reaction is visible. Body temperature 39°.2 C.

Forty-eight hours. Nose: Negative. Lip: Small, red, papular elevation on line of inoculation. Palate: Red line marks site of scratch. Body temperature 38° C.

Three days. Nose: A small gray crust is visible on the septum. Lip: 2 opaque, white elevations, 2 millimeters in diameter, are present, one at either end of the line of inoculation. Between these white spots runs a narrow white line. There is considerable reddening of the mucous membrane about the lesion. Palate: 2 small, opaque, white elevations, 1.5 millimeters in diameter, are joined by a narrow red line. Body temperature 39° C. Animal chloroformed and an autopsy done at once. Lesions at the site of inoculation as described above. The mucous membrane of the respiratory and digestive tracts examined and no evidence of a vaccine lesion found.

No. 85. Adult male *M. cynomologus.* Inoculated on the inner surface of the lip, on the nasal septum, and on the soft palate with vaccine virus No. 1. Body temperature 38°.8 C.

Twenty-four hours after the inoculation there is slight redness about the incision on the lip. The nose and the palate are negative. Body temperature 39° C.

Forty-eight hours. Nose: A brown crust is visible. Lip: The redness persists. Palate: 2 small yellow papular elevations are seen on the line of the inoculation. Body temperature 39° C.

Three days. Nose: Crust is still present. Lip: A small, opaque, white area, 1 × 2 millimeters, seated upon reddened mucous membrane. Palate: 2 opaque, white papular elevations, 2 millimeters in diameter, and elevated 1 millimeter above the general surface, surrounded by a pink flush. Body temperature 39°.2 C.

Four days. Nose: Small vesicle with a pink, peripheral flush. Lip: Opaque,

white area, 2 × 2 millimeters, with a distinct pink flush about it. Palate: 2 white elevations, 2 millimeters in diameter, flat-topped, and 1 millimeter in height, surrounded by an areola. Body temperature 39°.8 C.

Five days. Nose: Some degree of erosion of the mucous membrane of the septum at the site of inoculation. Lip: An area, 8 × 4 millimeters, presents an elevated, somewhat ragged, white edge, an eroded central portion, and a circumferential, pink flush. Palate: Elevated area, 8 × 3 millimeters, with a grayish-white center and a pink periphery. The nostril presents a vesicle which has spread from the mucous surface out upon the skin. Body temperature 39°.6 C.

Six days. Nose: Left nostril almost occluded by swelling of the mucous membrane and by yellow crusts. Lip: Lesion presents same features as yesterday. Palate: Lesion has enlarged somewhat and the central portion has a yellow color. Body temperature 39°.8 C.

Seven days. Body temperature 39°.5 C. Animal chloroformed. Nose: Along edge of nostril is a shallow excoriation, from which oozes a partly clear and partly cloudy fluid. The septum presents irregular, opaque, white elevations along the line of the inoculation. Lip: Lesion as before, save that loss of substance is more marked and there is some bleeding. Palate: Lesion as before. Viscera appear normal.

No. 86. Full grown female *M. cynomologus*. Inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39° C.

Twenty-four hours after the inoculation no evidence of a specific process is visible on inspection. Body temperature 39°.2 C.

Forty-eight hours. Nose: A narrow crust marks the site of the inoculation. Lip and palate negative. Body temperature 39° C.

Three days. Lesions show no change since yesterday. Body temperature 38° C.

Four days. Nose: White elevation at site of inoculation. Lip and palate negative. Body temperature 39° C.

Five days. Nose: An elevation 2 millimeters wide on septum near edge, with central loss of substance. Lip and palate negative. Body temperature 38°.2 C.

Six days. Nose: Left nostril almost closed by swelling of mucous membrane and yellow crusts. Inspection of interior not possible. Lip and palate negative. Body temperature 39° C.

Seven days. Nose: Edge of nostril presents a shallow erosion partly covered by a yellow crust. Lip and palate negative. Body temperature 39° C.

Eight days. Nose: Excoriation about nostril persists. Swelling of mucous membrane less marked. Lip and palate show no lesion. Body temperature 39° C.

Nine days. Nose: Lesion at edge of nostril from this time on heals like any vaccination of the skin. Animal allowed to survive. Twenty-two days after the first inoculation the monkey was inoculated again with vaccine virus No. 1. No lesion followed.

No. 87. Full grown male *M. cynomologus*. Animal inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39° C.

Twenty-four hours after inoculation the nose is negative, while the lip and palate show a red line on the mucosa at the site of the incision. Body temperature 39°.4 C.

Forty-eight hours. Nose: The inoculation scratch is now visible. Lip negative. Palate: Small red area at site of inoculation. Body temperature 38° C.

Three days. Nose: Mucosa pink, lesion not definite. Lip: A white line 3 millimeters long and 1 millimeter broad marks the site of the inoculation. Palate: Shows a white papular elevation 1 millimeter in diameter surrounded by a pink flush. Body temperature 39° C.

Four days. Nose: White elevation with a pink flush about it. Lip negative. Palate: Opaque, white, oval, elevated area, 2 × 3 millimeters, with a translucent center and a pink peripheral flush. Body temperature 39°.2 C.

Five days. Nose: Left side of the septum and the roof of the left nostril swollen; considerable mucous discharge. A translucent vesicle is present at the edge of the nostril. Lip: No lesion. Palate: A gray-white elevation, 2 × 3 millimeters, surrounded by a pink flush. Body temperature 40° C.

Six days. Nose: Left nostril all but occluded by a mass of yellow crusts springing from the septum and the upper border of the nostril. Lip negative. Palate: Oval area with an elevated, opaque, white edge, an eroded, pink center, and a distinct red areola. Body temperature 40° C.

Seven days. Nose: Lesions at edge of nostril extending and involving skin. Inspection of mucous membrane not possible on account of crusts and swelling. Lip negative: Palate: Lesion measures 6 × 4 millimeters, edge sharply circumscribed, elevated and gray-white, central portion gray and eroded. Body temperature 39°.5 C.

Eight days. Nose: Left nostril presents an opaque, white elevation, which bulges out from the septum and from the upper border of the orifice. The lesion at the edge of the nostril has extended somewhat on the skin surface. Lip negative. Palate: Lesion presents same features as yesterday. Body temperature 39° C.

Nine days. Nose: Lesion less extensive than before. Lip negative. Palate: Lesion presents same features as yesterday. Body temperature 39° C. Animal chloroformed. At autopsy no specific lesions found other than those at sites of inoculation.

No. 88. Adult male *M. cynomologus.* Inoculated inside of nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39° C.

Twenty-four hours after inoculation no reaction is visible. Body temperature 38°.6 C.

Forty-eight hours. Nose: A narrow, dark-red crust marks the line of inoculation. Lip: Mucous membrane reddened about scratch. Palate: An area 2 millimeters in diameter with an opaque white center and a deep red flush about it seen at the point of inoculation. Body temperature 38° C.

Three days. Nose: Narrow, brownish, depressed line bordered by an elevation the color of the mucous membrane. Lip: A line 1 millimeter wide, opaque-white in color, occupies the site of the scratch. About this line the mucous membrane is reddened. Palate: A narrow, irregular, white line bordered by an areola, the whole somewhat elevated, is visible at the site of inoculation. Body temperature 38°.2 C.

Four days. Nose: Mucous membrane injected but no definite lesion can be made out. Lip: An opaque, white area, 6 × 2 millimeters with small area of loss of substance and surrounded by a red areola. Palate: An oval area, 2 × 4 millimeters, slightly elevated, and seated on reddened mucous membrane. Body temperature 38°.5 C.

Five days. Nose: Some muco-purulent discharge from the left nostril. A white elevation with a pink border is visible on the septum. Lip: Opaque, white elevated area, 8 × 2 millimeters, with some superficial loss of substance and with a red areola. Palate: 2 lesions present, one 6 × 2 millimeters and one 2 × 2 millimeters. Both lesions are slightly elevated and present a gray center, a white periphery, and a red areola. Body temperature 38°.8 C.

Six days. Lesions same as yesterday save that they have extended somewhat. Body temperature 39° C. Animal chloroformed and an autopsy done at once. Specific lesions as described. No evidence of vaccinal lesions found in respiratory or digestive tract.

No. 89. Adult male *M. cynomologus*. Inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39°.5 C.

Twenty-four hours after inoculation the lip shows slight reddening of the mucous membrane about the scratch. The other sites are negative. Body temperature 39.°5 C.

Forty-eight hours. Nose and palate negative. Lip: 2 small, red, papular elevations, 2 millimeters in diameter, are present. About these the mucous membrane is reddened for a distance of 1 centimeter. Body temperature 39° C.

Three days. Nose: A small crust is visible at the site of inoculation. Lip: An irregular, opaque, white area, from 1 to 3 millimeters broad and 8 millimeters long, surrounded by a red flush, is present. Palate: 2 flat-topped, white elevations, 2 millimeters across and 1 millimeter high, seated on a slightly reddened mucosa, mark the site of the inoculation. Body temperature 39°.2 C.

Four days. Nose: Septum swollen and beset with yellow crusts. Lip: An irregular elevation from 2 to 4 millimeters wide and 12 millimeters long, dirty-white in color, and presenting superficial losses of substance. Lesion sharply circumscribed and surrounded by a deep-red areola. Palate: Lesions have increased in size. Body temperature 39°.6 C. Animal chloroformed. At autopsy no specific lesions found other than those at the site of the inoculations.

No. 90. Adult male *M. cynomologus*. Inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 40° C.

Twenty-six hours ofter the inoculation the animal was chloroformed. No reaction was visible at the site of inoculation. No visceral lesions were found at autopsy.

SUMMARY.

(1) *Macroscopic appearance of specific lesions.*—Lip: After 48 hours there appears a small, white or reddish papule, or simply a red area at the site of inoculation.

After 72 hours the mucous membrane is slightly elevated and opaque for a distance of 2 millimeters on each side of the scratch. There is a faint peripheral flush or areola.

After 4 days the opaque area has spread 1 or 2 millimeters, the areola is more marked, and the edge of the lesion is more or less sharply circumscribed. There may be some erosion of the opaque area and it always has a macerated appearance. From this time on the lesion spreads slowly, presents a rather sharp, elevated, white, opaque border, with a peripheral flush, the central portion being more or less eroded. There is no macroscopic evidence of vesicle formation or of crusting.

On the palate the lesion runs an identical course and begins to heal about the ninth day.

In the nose, the macroscopic appearances are unsatisfactory, owing to the swelling of the mucous membrane which prevents close inspection. When the inoculation is at all near the anterior nares, there is a decided tendency for the lesion to spread out over the skin about the nostril, where it takes on the characteristics of a skin inoculation.

(2) *Constitutional reaction.*—No general constitutional reaction was observed. The temperature reaction was not marked, although there was

slight elevation of the temperature on the fifth, sixth, or seventh day of the disease. No general exanthem was observed.

(3) *Histological examination of the specific lesions and of the viscera.*—Of the three foci chosen for inoculation, in two the lip and the palate yield similar microscopic pictures. In the nose two varieties of lesions are found, depending upon whether the lesion develops upon the portion lined by stratified or by columnar epithelium. In the former case the lesion is similar to that on the lip or palate.

In a general way the lesion on the palate which can be taken as a type of that on a stratified mucosa epithelium suggests a lesion on a hairless skin from which the crust and the other superficial parts have been removed. When we examine in detail lesions of various durations we find a close similarity in them to the vaccine lesion on the skin, both in the cell changes and in the histogenesis of the lesion. For forty-eight hours after the inoculation the only change demonstrable in a small cleft in the submucous connective tissue which is filled by blood and fibrin, with an occasional polynuclear leucocyte. A few leucocytes are also to be found in and about the neighboring vessels and in the overlying epithelium which has closed over the inoculation wound. Later lesions show swelling and degeneration of the epithelial cells over a small area, which increases in size from day to day as the lesion spreads. If we study a lesion of 5 days' duration we find at the periphery some swelling of the individual epithelial cells, which gives place, as we approach the center of the lesion, to various degenerations. In this thickened epithelium at the edge of the lesion we find fluid collecting between the cells in such a manner as to form minute chambers similar to those in the periphery of a skin lesion of the same duration. This is the only approach to vesicle formation presented by these lesions. In the center of the lesion the epithelial cells are quite unrecognizable, and the space is filled by fibrin, leucocytes, and cell detritus. There is often a rather sharp line of demarcation between the swollen but comparatively normal epithelium which borders the lesion and the degenerated central portion. Occasionally islands of pathological, but still recognizable, epithelial cells are found in the necrotic area.

The submucous tissue presents a complicated cell picture. The blood vessels are more or less prominent, in part on account of swelling and proliferation of their endothelial cells and in part from the large number of polynuclear leucocytes within them and migrating through their walls. The connective-tissue cells are more or less swollen and are frequently undergoing mitosis. A considerable number of eosinophile leucocytes are frequently present. Polynuclear leucocytes occur in large numbers but do not form such a prominent part of the picture as they do in the skin lesion. Besides these readily identified cells there are many mononuclear cells present which are not so easily distinguished. The majority

of them seem to belong to the lymphoid-plasma cell series, while some are probably of endothelial origin.

In the older lesions evidence of repair is found. The epithelial cells grow in from the epithelium at the periphery of the lesion and upward from the ducts of the glands. There is active, new formation of blood vessels and of connective tissue beneath the lesion.

In the portion of the nose covered by columnar epithelium, the process is somewhat different from that described above. We find large areas denuded of epithelium and a marked reaction of an inflammatory sort in the tissue beneath. The lack of coherence between the columnar epithelial cells in this situation prevents any marked thickening at the edge of the lesion.

The protoplasmic phases of *cytoryctes variolæ* were present in the epithelial cells in lesions of all durations and in all situations. Infected endothelial cells were demonstrable in one lesion. They occurred in a capillary beneath a lesion of six days' duration on the stratified epithelium of the nose.

DISCUSSION.

The foregoing experiments show that an inoculation upon the nasal, buccal, or oral mucous membrane of the monkey with vaccine virus produces a characteristic, self-limiting lesion. Histological study of these lesions shows them to be similar to those produced on the skin by vaccination. The differences observed in the vaccine lesion on the mucous membrane from that on the skin are readily explained by the physical conditions at the site of inoculation. The vaccine lesion on the mucous membrane shows no crust or vesicle. The absence of these characters is undoubtedly due to the fact that the epithelium at this site of inoculation does not possess a horny layer, and also to the fact that the surfaces are constantly rubbing one against the other and are bathed with fluid. We have seen that small collections of fluid between the swollen and degenerated cells of the lesion may take place in the vaccine lesions of the mucous membrane. This process is the same as that seen in the peripheral portions of a developing vaccine lesion on the skin. However, as soon as a considerable amount of fluid collects the tendency is for it to escape on the surface, and so no large vesicle forms. The reaction in the tissue beneath the lesion is similar in kind to that beneath a vaccine lesion of the skin, but the process is less in degree. If we assume that the reaction in the corium beneath the skin vaccination is due to substances absorbed from the specific process in the epidermis, it is to be expected that the reaction would be less intense beneath a vaccine lesion on a mucous membrane. In the latter situation the greater part of the products of the process in the epithelium must escape on the surface. The mildness of the constitutional reaction and the slight degree of fever which these animals present is doubtless due to the latter cause. The

constant presence of the cytoplasmic phases of *cytoryctes variolæ* in the epithelial cells of the lesion and the absence of nuclear phases is consistent with the hypothesis that the latter are peculiar to variola, for as we will show later, the epithelial cells of the mucous membrane present many examples of the nuclear phases of the organism when variola virus is used for inoculation.

CONCLUSIONS.

(1) Vaccination of the monkey, *M. cynomologus*, upon the nasal, oral, or buccal mucous membrane gives rise to a true vaccine lesion similar to that which follows vaccination of the skin.

(2) The vaccine lesion on the mucous membrane shows certain differences from that upon the skin, but these differences are explained by the physical conditions at the *locus* of inoculation.

(3) The presence of protoplasmic phases of *cytoryctes variolæ* and the absence of nuclear phases of the organism in the vaccine lesion on the mucous membrane is consistent with the hypothesis that the former cycle is associated with the lesions of vaccinia and that the latter do not occur in such lesions, being peculiar to variola.

PART II.

STUDIES UPON EXPERIMENTAL VARIOLA IN MONKEYS (MACACUS CYNOMOLOGUS AND M. NEMESTRINUS) AND IN THE ORANG-UTAN (SIMIA SATYRUS).

1. VARIOLA INOCULATA FOLLOWING INOCULATION OF THE SKIN OF THE MONKEY.

In these experiments particular attention was paid to the evolution of the specific lesion at the site of the inoculation, to the general exanthem, and to the constitutional reaction of the animal. Material was collected for the histological study of the primary lesion, of the exanthem, and of the internal organs.

Technique.—The different strains of virus used were obtained from cases of smallpox occurring among the European and native population of Manila and of the provinces. So far as circumstances permitted, the virus was kept on ice from the time of collection until used for inoculation.

In some experiments the contents of the pustule or the dry disk was used, but as a rule the contents of the unclouded vesicle was selected for inoculation. Each strain of virus, except one, was inoculated at least once upon the skin of a fresh monkey to test its potency. The different strains used were as follows:

No. 21. Collected April 6, 1904, from a case of severe *variola vera* in a native male, aged about 20 years, on the twelfth day of the disease.

No. 52. Collected May 4 from a case of severe *variola vera* in an American negro in Bilibid Prison, aged about 30 years, on the tenth day of the disease.

No. 167. Collected from a case of severe *variola vera* in a native male, aged about 20 years. The contents of the unclouded vesicle was collected on the eighth day of the disease, August 6, and again on the following day. The contents of the pustules was collected on the fifteenth day and the disks from the palms and soles on the nineteenth day of the disease.

No. 199. Collected on September 18 from a case of severe *variola vera* in an American infant on the ninth day of the disease.

No. 200. Collected on September 17 from a case of severe *variola vera* in a Spanish-Filipino infant on about the ninth day of the disease.

No. 252. Collected October 7 from a case severe *variola vera* in a native infant, at autopsy, on about the tenth day of the disease.

No. 307. Collected on October 23 from a case of severe *variola vera* in an adult native male on about the ninth day of the disease.

No. 325. Collected November 3 from a case of mild *variola vera* in a native boy, aged 8 years, on about the tenth day of the disease.

No. 326. Collected November 6 from a case of severe *variola vera* in a native boy, aged 12 years, on about the eighth day of the disease.

No. 327. Collected on November 9 from a case of severe *variola vera* in a native girl, aged 11 years, on about the ninth day of the disease.

Method of inoculation.—The introduction of the contagion into the animal was effected by making a number of separate shallow scratches with the point of a scalpel on the previously shaved and cleaned skin of the abdomen, and rubbing the virus into the wound with the back of the instrument. The inoculations were placed at least 2 centimeters apart. The usual precautions were observed to avoid the introduction of extraneous matter. An anaesthetic was employed whenever discomfort might otherwise be caused to the animal.

The animals were observed daily during the course of each experiment. The appearance of the lesions to the naked eye at the sites of inoculation was recorded, together with the occurrence and evolution of the exanthem, the constitutional reaction, the body temperature taken per rectum, and the reaction of the lymph nodes.

Material for histological study was obtained either by excision of the lesions during the course of the experiment, or by autopsy when the animal was killed. All tissues were fixed in Zenker's fluid for twenty-four hours, washed in running water over night, and then hardened by passing through alcohols of graded strength. Tissues were embedded by the chloroform-paraffine method. Sections were cut on the Minot microtome of a thickness of from 3 to 6 microns, and were stained in a variety of ways.

In certain experiments the immunity of the animal, resulting from the first inoculation, was tested by subsequent skin inoculations with vaccine or variola virus.

This section is based upon the study of sixty-five monkeys inoculated on the skin with variola virus, of which the following experiments are selected to be given in detail.

1. *Clinical course of the disease.*

No. 114. Adult, male, *Macacus cynomologus*. Monkey was inoculated in 12 places on the skin of the abdomen with virus No. 167 (vesicle contents). Body temperature 37°.6 C.

Twenty-four hours after inoculation the scratches show a narrow, dry crust, about which the skin is opaque and slightly elevated for a distance of 1 or 2 millimeters. Body temperature 39°.2 C.

Forty-eight hours. The skin for a short distance about the crusts is white, but this area fades, without a definite line of demarcation, into the surrounding normal skin. Body temperature 38°.2 C.

Three days. The elevated skin about the crust is pink for a distance of 2 millimeters. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. The lesions are present as rounded, pink elevations with fairly definite borders and surmounted by delicate yellow crusts. The lesions average

7 millimeters across. In some there is a narrow translucent zone immediately around the crust. Axillary lymph nodes somewhat enlarged and firm. Body temperature 40° C.

Five days. The central crust is depressed, and is surrounded by a definitely elevated vesicular ring, which is bordered externally by a red areola, fading outwards into normal skin. There is much œdema of the subcutaneous tissue beneath the lesions, producing a broad indurated base on which the lesions are individually prominent. The lesions average 8 millimeters in width. Axillary lymph nodes distinctly enlarged and hard. Body temperature 41°.5 C.

Six days. Both the crust and the vesicle have increased in extent. The contour of the lesion shows an abrupt elevation in the zone of the areola. Average width, 10 millimeters. A few small red papules are noted on the shaved area of the abdomen near the belt line. Axillary lymph nodes enlarged and hard. Body temperature 40°.8 C.

Seven days. The lesion presents as a flat-topped elevation with a central brown crust and an opaque vesicular ring. Average width 8 millimeters. The lesions are less prominent to-day, owing to the subsidence of the subcutaneous œdema. Two of the papules noted near the belt line have increased somewhat in size. Axillary lymph nodes as before. Body temperature 40° C.

Eight days. Primary lesions are beginning to undergo involution, the crust is spreading at the expense of the vesicular ring. Subcutaneous œdema has almost disappeared. On the face there are a dozen or more papules and vesicles from 2 to 4 millimeters in diameter. Similar lesions are present on the abdomen, the scrotum, the inner aspect of the thighs, and on the palms. Axillary lymph nodes as before. Body temperature 40° C.

Nine days. The vesicular element in the primary lesions has been obliterated by the spreading of the central crust. The lesions of the exanthem are present as filled out vesicles, some of which have opaque, white or yellow contents, and all are surrounded by a bright-red areola. An abundant eruption is noted on the tail and the skin about its base. Axillary lymph nodes slightly enlarged and hard. Body temperature 39°.5 C.

Ten days. Many of the primary lesions have been scratched and are present as shallow ulcerations over which the epithelium is spreading from the edge. The lesions no longer present specific characters. The lesions of the exanthem are dry and crusted. The eruption is noted to-day on the soles and on the dorsal aspect of several of the fingers and toes. Axillary lymph nodes of almost normal size but still firmer than normally. Body temperature 38°.5 C.

From this time on the specific lesions healed without complications. Material was collected at intervals for the histological study of the primary lesions.

No. 115. Half-grown male, *Macacus cynomologus*. Inoculated in 12 places on the abdomen with virus No. 167 (vesicle contents). Body temperature 38° C.

Eighteen hours after the inoculation a narrow, brown crust, surrounded by a sharply circumscribed, elevated, opaque, white area 5 millimeters across, marks the site of the inoculation. Body temperature 39°.4 C.

Forty-eight hours. The skin about the crust is elevated and pink for a distance of 2 millimeters. Body temperature 37°.8 C.

Three days. The elevated area has increased in extent and is distinctly red in color. Axillary lymph nodes slightly enlarged. Body temperature 38°.8 C.

Four days. Immediately about the crust there is a distinct translucence of the skin. Average width of lesions, 7 millimeters. Axillary lymph nodes distinctly enlarged. Body temperature 39°.5 C.

Five days. The lesion is present as a rounded elevation surmounted by a narrow, brown crust, about which there is a distinct vesicular ring, translucent

near the crust and shading insensibly into a pink zone, which fades out into the surrounding normal skin. Considerable subcutaneous œdema. Average width of lesions, 8 millimeters. Axillary lymph nodes distinctly enlarged and hard. Body temperature 41°.5 C.

Six days. The central crust is surrounded by a narrow, opaque, white vesicle on a pink elevation, measuring from 9 to 10 millimeters across. Axillary lymph nodes as before. Body temperature 39°.8 C.

Seven days. The lesions present as flat-topped, sharply circumscribed elevations, from 9 to 10 millimeters in diameter. The central crust, somewhat depressed, is surrounded by an elevated vesicular ring which is opaque-white in color. On the upper lip, the abdomen, the arm, and the scrotum are seen small, red, papular elevations. Axillary lymph nodes as before. Body temperature 39°.8 C.

Eight days. Yellow, turbid fluid oozes from beneath the central crust. The vesicular ring has been obliterated in places by the spreading of the crust. The lesions of the exanthem are somewhat larger and vesicular. Three new lesions have appeared on the face and one in the groin. Lymph nodes as before. Body temperature 39°.5 C.

Nine days. The vesicular zone of the primary lesion has been entirely obliterated. The subcutaneous œdema has almost disappeared. To-day an exanthem is noted at the edge of the nostril and in the vestibule. The tail and the skin at its base present numerous small papules and vesicles, each surrounded by a distinct areola. Axillary lymph nodes enlarged and hard. Body temperature 39°.5 C.

Ten days. Primary lesions are healing and present no specific characters. The lesions of the exanthem have dried and are healing. One eruptive lesion found on the sole of the foot and several on the dorsal aspect of the toes. Axillary lymph nodes of almost normal size but still firm. Body temperature 39°.5 C.

No. 116. Young, adult male, *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with virus No. 167 (vesicle contents). Body temperature 38°.5 C.

Eighteen hours after the inoculation the skin about the scratch is slightly elevated and opaque for distance of 2 millimeters. Body temperature 38°.6 C.

Forty-eight hours. The site of inoculation is marked by a narrow, yellow crust surrounded by a pink elevation 3 millimeters in width. Body temperature 37°.8 C.

Three days. The primary lesion as before, save that the elevation is more marked and the color deeper. Axillary lymph nodes slightly enlarged. Body temperature 40° C.

Four days. The brown, central crust is seated upon a pink elevation 6 to 10 millimeters across, which fades out into the normal skin. Immediately about the crust there is a narrow zone of translucence suggesting vesicle formation. A marked subcutaneous œdema renders the lesions prominent. Axillary lymph nodes enlarged and hard. Body temperature 40° C.

Five days. Primary lesion as before but it has increased in size. Axillary lymph nodes markedly enlarged and hard. Body temperature 41° C.

Six days. The crust is surrounded by a definite vesicular ring, which in turn is surrounded by a dull pink areola. Average width of lesions, 9 millimeters. Axillary lymph nodes as before. Body temperature 39°.5 C.

Seven days. The primary lesions present as circumscribed elevations with a central, depressed, brown crust, an elevated, opaque-white, vesicular ring, and a bright-red areola. Average width of lesions 9 millimeters. On the face, the abdomen, the scrotum, the thighs, and the arms are numerous small papules or vesicles, each surrounded by a red areola. Axillary lymph nodes as before. Body temperature 39°.8 C.

Eight days. Primary lesions no longer show a vesicle. Many of the papules have become vesicles and the vesicles pustules. There is some increase in size of the lesions of the exanthem. Axillary lymph nodes as before. Body temperature 40° C.

Nine days. Some subcutaneous œdema persists beneath the primary lesions, which, however, have lost their specific character. The exanthem is beginning to dry on the face. Many papules and vesicles, not noted before, are visible on the tail and on the skin at its base. Axillary lymph nodes enlarged and hard. Body temperature 39°.5 C.

Ten days. The primary lesions are healing. Eruptive lesions found to-day on soles and palms. Axillary lymph nodes still enlarged and hard. Body temperature 39°.6 C.

No. 117. Adult male, *Macacus cynomologus*. Inoculated with same virus and in the same way as the preceding animals. Body temperature 37°.8 C.

Eighteen hours after inoculation a narrow yellow crust is seen upon an opaque, slightly elevated area. Body temperature 39°.2 C.

Forty-eight hours. The elevation and opacity have extended somewhat. Body temperature 37°.2 C.

Three days. The lesion presents a linear crust on a slightly elevated, pink area 4 millimeters across. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. About the crust is a narrow translucent zone which merges with the pink elevation, which in turn fades off into the surrounding skin. Average width of lesions, 7 millimeters. Axillary lymph nodes distinctly enlarged and hard. Body temperature 39°.5 C.

Five days. A distinct but narrow vesicle is evident around the crust. Considerable œdema beneath the lesions. Areola well marked. Axillary lymph nodes as before. Body temperature 40°.5 C.

Six days. Certain of the lesions show a typical development presenting a crust, a definite vesicle, and an areola, the whole lesion being sharply elevated from the surrounding skin. Other lesions show departure from the normal type in that the vesiculation is less marked and the lesions are not as sharply circumscribed. Lymph nodes enlarged and hard. Body temperature 40°.2 C.

Seven days. Some lesions typical, others show a spreading of the crust without macroscopic evidence of vesicle formation. Average width of lesions 9 millimeters. On the face, abdomen, scrotum, inner aspect of thighs, axillæ, and arms are numerous pink, papular elevations 2 millimeters in diameter, some of which present translucent central points. Axillary lymph nodes as before. Body temperature 39°.6 C.

Eight days. Certain of the primary lesions show remnants of the vesicle at the edge of the spreading central crust. The lesions of the exanthem have increased in size and the contents of many of the vesicles has become cloudy. New eruptive lesions are present on the abdomen. Axillary lymph nodes as before. Body temperature 39°.8 C.

Nine days. The primary lesions show a narrow zone of translucent epithelium close to the crust. This appearance suggests the beginning of the healing of the lesion by the ingrowth of new epithelium rather than vesicle formation. Lesions of the exanthem have dried up with or without rupturing. Axillary lymph nodes slightly enlarged but firmer than normal. Body temperature 39°.5 C.

Ten days. The healing of the primary lesions is well under way, and they have lost all specific character. To-day an exanthem is noted in the region about the ischial tuberosities and beneath the tail. Eleven eruptive lesions found in the palms and soles and many are also present on the fingers and toes. Axillary

lymph nodes slightly enlarged and hard. Body temperature 39° C. Specific lesions healed without complications.

No. 118. Adult, male, *Macacus cynomologus*. Inoculated with the same virus and in the same manner as the previous animals. Body temperature 38°.8 C.

Eighteen hours after inoculation. Some elevation and opacity is evident about the scratch. Body temperature 38°.5 C.

Forty-eight hours. Elevation and opacity more marked. Body temperature 37°.4 C.

Three days. Lesion presents as a narrow, yellow crust on a pink elevation 5 millimeters across. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. A narrow zone of translucence is visible near the central crust. The lesion is roundly elevated, of a pink color, and merges without sharp line of demarcation with the surrounding skin. Average width of lesion, 7 millimeters. Considerable subcutaneous œdema. Axillary lymph nodes enlarged. Body temperature 39°.6 C.

Five days. A definite vesicular ring surrounds the crust and is surrounded in turn by a pink areola. The lesion is not sharply circumscribed. Axillary lymph nodes enlarged and hard. Body temperature 41°.2 C.

Six days. The vesicular ring has become opaque and has increased in extent. There is some diversity in the size of the lesions. Axillary lymph nodes as before. Body temperature 40°.6 C.

Seven days. The central crust is surrounded by an elevated ring in which evidence of vesiculation can be made out in places. The edge of the lesion is sharply circumscribed and rises abruptly from the surrounding skin. A definite pink areola is present. Average width of lesions, 9 millimeters. On the face, inner aspect of arms, thighs, and abdomen are red, papular elevations 2 millimeters in diameter. Some of these present a minute translucent center. Axillary lymph nodes as before. Body temperature 40°.2 C.

Eight days. Involution of primary lesions has begun. The vesicular ring has entirely been obliterated by the spreading of the central crust. The subcutaneous œdema has disappeared. The lesions of the exanthem have increased in size and are definitely vesicular. Some new papules are present today upon the abdomen and face. Axillary lymph nodes of normal size but firmer than normal. Body temperature 40°.2 C.

Nine days. Primary lesions beginning to heal. The exanthem is dry and no new lesions have appeared. Axillary lymph nodes as before. Body temperature 39°. C.

Ten days. Primary lesions not notable. Eruptive lesions, not noted before, are present on the palms. Axillary lymph nodes as before. Body temperature 39° C.

No. 170. Adult male, *Macacus cynomologus*. Inoculated in 6 places on the skin of the abdomen with a suspension of pulverized disks (virus No. 167) in sterile salt solution.

Three days. An opaque elevation 3 millimeters across is present at the side of inoculation. Body temperature 39° C.

Five days. Lesion presents a small crust on a pink elevation 5 millimeters across. A narrow zone of translucence borders the crust. Body temperature 39° C.

Six days. The central crust is depressed and about it there is irregular vesicle formation. The lesions vary much in size. Body temperature 40° C.

Seven days. Primary lesions have increased slightly in extent. Considerable subcutaneous œdema is present. Body temperature 39°.5 C.

Eight days. Some of the primary lesions show a distinct but irregularly developed vesicular zone. Near anus is a single vesicle 2 millimeters across surrounded by a red areola.

Nine days. The central crust has encroached upon and partly obliterated the vesicular zone. Single small vesicles are present on the arm, the thigh, beneath the tail, and on the ankle.

Ten days. Primary lesions healing. No new exanthem.

No. 171. Inoculated with the same virus and in the same manner as the preceding monkey.

The evolution of the primary lesion was delayed and the vesiculation irregular, as in the previous experiment. No general exanthem was observed. The temperature reaction was indefinite.

No. 164. Half grown male, *Macacus cynomologus*. Inoculated in 6 places on the skin of the abdomen with a suspension of pustular contents [(virus No. 167), dried with lycopodium powder] in sterile salt solution.

Twenty-four hours after inoculation there is slight elevation and opacity about the scratches.

Subsequent to this, for a period of 5 days after the inoculation, there is no evidence of a process.

Six days. Lesions present a narrow crust on a pink elevation. The appearance is the same as that seen on the third day after an inoculation with fresh vesicle contents.

Seven days. The lesion has increased somewhat in extent and is sharply circumscribed.

Eight days. An irregular vesicular zone is developed about the crust.

Ten days. Vesiculation no longer apparent. But slight induration can be made out in the lesion. No general exanthem was observed either before or after this date. The animal was subsequently inoculated with vaccine virus No. 1, but failed to react.

No. 194. Young male, *Macacus nemistrinus*. Inoculated in 12 places on the skin of the abdomen with virus No. 199. Body temperature 38°.8 C.

Twenty-four hours after inoculation there is slight elevation and opacity about the scratch. Body temperature 37°.8 C.

Forty-eight hours. Marked elevation and opacity around the linear crust. Body temperature 39°.1 C.

Three days. There is marked subcutaneous œdema beneath the lesion. The elevation about the crust is distinctly pink in color. Body temperature 38°.9 C.

Four days. The lesion consists of a rounded, pink elevation 5 millimeters across, surmounted by a crust about which is a narrow zone of translucence, the whole lesion being underlaid by a broad indurated area of subcutaneous œdema. Body temperature 39°.3 C.

Five days. An irregular development of the vesicular ring is apparent in certain of the lesions. Body temperature 39°.5 C.

Six days. Many of the primary lesions have been scratched by the animal and are present as excoriated areas surrounded by a red elevation and seated upon a broad indurated base. Body temperature 39°.6 C.

Seven days. Primary lesions as before. A profuse exanthem consisting of small, red papules and vesicles is present upon the face, trunk, and extremities. Body temperature 38°.7 C.

Eight days. The primary lesions have crusted and in places there is slight vesicle formation. Several new papules and vesicles are present on the face and thighs. One small vesicle is visible on the hard palate. Body temperature 38° C.

Animal killed and autopsy done at once. Material saved for histological examination of specific lesions and of the viscera. Axillary lymph nodes enlarged and red. On section much blood-stained fluid exudes from the cut surface.

II. *Histological examination.*

The primary lesion 18 hours after inoculation: Microscopic examination of sections from the site of the inoculation shows a solution of continuity which involves the epithelium and may or may not extend into the corium beneath. The defect is filled by an exudate composed of polynuclear leucocytes which lie in a meshwork of fibrin. On the surface the elements of this exudate are fused into a crust. On either side of the defect the epithelial cells of the rete are more or less swollen, and some present clear, circular areas in their protoplasm, suggesting hydropic degeneration. The nuclei of these cells are swollen and the chromatin tends to collect in masses. Polynuclear leucocytes are present in considerable numbers in and around the vessels of the corium and in the connective tissue beneath the defect. The leucocytes are streaming into the defect and into the adjacent epithelium.

Forty-eight hours. The sections present the same general characters as those from the earlier lesions. The defect in the epithelium is sometimes obliterated in such a manner that a layer of epithelium is interposed between the exudate which has collected beneath the crust and the injury to the corium. The polynuclear leucocyte infiltration is more intense. The epithelial cells of the lower layers of the epidermis about the line of inoculation show more marked degeneration, and the cells are frequently separated one from another by fluid.

Three days. The crust has increased in thickness and, with the destruction of the epithelial cells in the central line of the lesion, may fuse with the corium. The epithelial cells of the lower layers adjacent to the crust show various forms of degeneration. Some present ballooning degeneration, and the accumulation of fluid between the cells is more evident than in the earlier lesions. In places, vesicle formation is foreshadowed by the appearance of small, irregular cavities formed either by the accumulation of fluid between the cells or by the bursting of hydropic cells. The corium is the site of a definite reaction, shown by an enlargement of the endothelial cells of the blood and lymph vessels. These enlarged endothelial cells may contain cytoryctes. There is a marked polynuclear leucocyte infiltration of the corium and the epithelium about the crust. In some cases a mass of leucocytes, with granular precipitate and fibrin, is found in the corium just beneath the center of the lesion. The cellular sheaths of the hair follicles show changes similar to those seen in the cells about the crust, and an abundant polymorphonuclear infiltration is present. Some lesions of this age show definite vesicle formation such as will be described later.

Four days. Lesions of this age may present vesicular cavities under the lateral expansions of the crust. In some lesions the whole crust is elevated and the lateral vesicles communicate with one another. The epithelium at the sides of the lesion and below the lateral portions of the vesicle is more or less swollen and shows various degenerations, the most marked forms of which are found in those cells nearest the center of the lesion. The epithelium is completely destroyed in the axis of the lesion. The corium beneath shows proliferation of the endothelial cells of the blood vessels and of the lymphatics, together with an enlargement of the connective-tissue cells. A polymorphonuclear leucocyte infiltration is apparent in the corium and in the thickened epithelium about the vesicle. In some lesions the contents of the vesicle shows a predominance of polymorphonuclear leucocytes under the crust, while the outer portions are all but free from these cells, containing only granular precipitate and fibrin.

Five days. The topography of the lesion at this stage shows considerable variation, due to differences in the extent of the vesicle formation. The typical

picture is similar to that in the four-day lesion with some increase in the extent of the vesicle and in a more marked reaction in the corium beneath the lesion. The polynuclear leucocyte infiltration of the corium is less intense, but the vesicle cavity shows a more even distribution of the pus cells. The reaction of the corium is more marked, being shown by the presence of œdema, necrosis, and an increase in the cellular content of the tissue. Aside from the polynuclear leucocyte the dominant element is a large cell with a vesicular nucleus surrounded by an abundant reticular protoplasm. These cells show a great variation in form, some being rounded, others irregular, the outline seemingly being conditioned by the space in which the cell lies. Many of these cells are found just outside of the capillaries and in the lymph spaces of the tissue, and by comparison of their morphology and staining reaction with that of the swollen endothelial cells, *in situ* in the capillaries and in the lymph spaces, it seems certain that they are identical with them. Many of these cells are phagocytic and in some mitosis is seen. In certain of these cells early stages of the cytoplasmic phases of *Cytoryctes variolæ* are present. In addition to these endothelial cells, which have often wandered for some distance from their place of origin, a certain number of elements are found of the lymphoid and plasma cell series. The connective-tissue cells all through the corium about the lesion are swollen, and in some mitosis is in progress. The nerve bundles are frequently invaded by polymorphonuclear leucocytes. The cellular reaction of the corium is shared by the adjacent subcutaneous tissue and extends for a considerable distance from the line of inoculation. Necrosis is seen in the tissue beneath the center of the lesion, the cells losing their basic affinity and undergoing more or less fragmentation or solution. Many deeply stained spherules of various sizes are scattered through the necrotic tissue, evidently the nuclear fragments of polynuclear leucocytes.

Six days. The general relations of the vesicle cavities and the crust remain as before, although the outer limits of the vesicle often extend beyond the limits of the crust, being roofed by a layer of cornified epithelium which sweeps downward to form a part of the lateral wall of the cavity. In some instances the beginning of vesicle formation, shown by collection of fluid between tne cells, is apparent a short distance beyond the outer limits of the main vesicle. A similar condition is met with in the depths of the cellular sheaths of the hair follicles. The fusion of the middle portion of the crust with the underlying corium is often apparent, although in many places a collection of leucocytes is present here which is continuous with the lateral vesicles and with the purulent focus which forms in the corium along the line of inoculation. In such a lesion a roughly T-shaped cavity can be made out, the vertical portion being filled with leucocytes and extending from the crust for a variable distance into the corium, while the cross arm has for its extremities the vesicular cavities which extend laterally under the crust. The vertical portion of this T can often be traced to the focus of necrosis which lies in the lower layers of the corium and the upper portion of the subcutaneous tissue. The reaction of the corium is very marked at this stage. Study of the outer limits of the necrotic area shows that the large cells, which we believe to be endothelial in origin, seem to be resistant to the agent which is causing the necrosis. The large cells are frequently to be seen in an apparently normal condition in an area where all other cellular elements have been destroyed. It is hard to determine whether this apparent immunity from destruction is due to qualities of the cell or whether it is because the cells have migrated into the necrotic area. In one instance a small capillary was found in the corium near the necrotic area line by swollen endothelial cells, many of which were infected with early stages of the cytoplasmic phases of *cytoryctes variolæ*.

Seven days. The lesions of this age and those collected later show a progres-

sive healing of the process. The vesicle soon disappears and the polynuclear leucocyte infiltration of the whole field becomes less intense and eosinophiles appear. The epithelium grows inward under the crust and is joined by that which comes from the proliferation of cells of the hair sheaths. The blood vessels of the corium, send out prolongations, and the usual phenomena of repair dominate the picture.

The exanthem: Sections of lesions collected on the first day of the appearance of the exanthem show a vesicular cavity of variable size in the epidermis. This cavity is roofed by a layer of cornified epithelium and laterally and below is surrounded by thickened epithelium. The cells adjacent to the cavity show varying degrees of degeneration. In every case the layer of the *rete* which forms the floor of the vesicle is wanting at one or more points, so that the cavity in the epidermis is in communication with the corium. The blood vessels and the lymphatics of the corium beneath the lesion present marked changes. These are in part due to the migration of polynuclear leucocytes which is going on from the vessels into the corium, the vesicle, and the epithelium about it. Besides this purely exudative phenomenon the endothelial cells of the capillaries and of the lymphatics show marked swelling and some proliferation. The normal relationships of structure in the corium are much disturbed by this combination of exudation, proliferation, and swelling. A careful search of such areas failed to show any cytoryctes, although they were numerous in the cells of the *rete* which formed the floor of the vesicle.

Sections from the exanthem collected later in the evolution of the lesion show an increase in the size of the vesicular cavity, but no characters other than those found in the early lesions.

The study of sections from the primary lesions and of the exanthem in *M. nemestrinus* show variations in degree but not in kind from the picture seen in the corresponding lesions in the Philippine monkey. The reaction in the subcutaneous tissue and in the corium is more marked and œdema plays a more important rôle. The vesicle is not so well developed, but is similar in all fundamental characteristics to that in *M. cynomologus*.

Axillary lymph nodes: The sinuses are dilated, sometimes to a high degree. The cell content of the sinuses shows various deviations from the normal. The most prominent character is an increase in the number of endothelial cells. These cells increase greatly in size, become free in the sinuses, and show marked phagocytic properties. In the nodes from monkeys killed on the sixth and eighth days of the disease the included cells are in part red blood corpuscles and in part polynuclear leucocytes. Later the polynuclear leucocytes are the common inclusion, though lymphoid and other cells may occasionally be found within the phagocytes. Besides the endothelial phagocytic cells, red blood corpuscles, and polymorphonuclear leucocytes are found in considerable numbers free in the sinuses. The latter cells predominate in the nodes from animals killed on the ninth day of the disease and later. Eosinophile cells are frequently encountered, but do not as a rule occur in such numbers as do the polymorphonuclear leucocytes.

The follicles show many phagocytic endothelial cells, singly or in small groups, scattered through their substance. In nodes from animals killed on the eighth day of the disease, small areas of hemorrhage were frequently found. In these areas red blood corpuscles were present in the follicular tissue about a small capillary, many of them having been taken up by phagocytes. In nodes collected later in the disease large phagocytic endothelial cells were demonstrable, whose whole cell body was crowded with red blood corpuscles in various stages of dissolution. In one node, collected on the eighth day of the disease, masses of eosinophile leucocytes were present in the follicles and in the sinuses. These cells differed from the usual eosinophile cells in that the granules were elongated.

Eosinophile cells having round, or slightly oval, granules were also present. The cells with the long granules differed from the usual eosinophile cells in having a more distinct cell membrane than the regular type of eosinophile. In this respect they conformed more closely to the type of the polymorphonuclear leucocyte.

Bone marrow and testicle: As these organs present the only specific visceral lesions of smallpox in man they were scrutinized with care in our variolated monkeys. No macroscopic evidence of focal lesions in these organs were seen at autopsy, and the bone marrow from four cases and the testicle from twelve were negative in this respect when studied microscopically.

Liver, spleen, kidney, and lung: No pathological process was demonstrable either macroscopically or microscopically in these organs.

SUMMARY.

1. (*a*) *The macroscopic appearance of the primary lesion in Macacus cynomologus.*—Twenty-four hours after inoculation there is some elevation and some opacity of the skin about the scratch. The process has rarely a lateral extent of more than 2 millimeters.

After 48 hours the appearance is usually the same.

After 3 days the elevation is more marked and the opacity gives place to a pink or red appearance. So far as can be discovered by the naked eye, the specific process may now be said to have begun. Previous to this the reaction has been in no way different from that which follows a simple scratch, without the introduction of virus.

After 4 days the lesion usually attains a breadth of 6 or 7 millimeters and is present as a distinctly pink or red elevation, which is firm to the touch and bears on its summit a narrow crust which has its origin in the drying of the serum exuding at the time of inoculation. However, the crust has somewhat increased in extent. At this time the skin about the crust is more or less translucent. It is often impossible to say whether or not this translucence is, at this time, the site of a definite vesicle.

After 5 days the lesions show the characteristics of a pock, being resolvable into rather distinct zones which correspond with those distinguishable in the microscopic sections. Going from the center of the lesion toward the periphery, we recognize in turn—first a crust, second a vesicular ring, third a zone of elevation and hyperæmia. The lesion now has a lateral extent of from 6 to 8 millimeters. The whole lesion is more or less elevated upon a broad indurated base, due to œdema of the skin and subcutaneous tissues.

After 6 days the picture is the same, save that each of the zones has extended peripherally. At about this time the profile of the lesion undergoes a change in that it loses its flowing outline as a more or less hemispherical elevation, and takes on a flat-topped, plateau-like appearance. In going from the center outward we pass along the fairly flat crust, then over the vesicular ring, which attains a greater elevation than the crust, forming a rampart, and then come to an abrupt declivity where the zone of hyperæmia or areola extends into normal skin.

After 7 days the crust and the vesicular ring will be seen to have extended somewhat, but the former has encroached more or less upon the area occupied by the latter. The subcutaneous œdema is less marked.

After 8 days the lateral excursion of the lesion has definitely ceased and involution begins. This process of involution is evidenced by the spreading of the crust so that it finally occupies all the territory held by the vesicular ring. At the same time the zone of hyperæmia fades the subcutaneous œdema disappears, and the lesions eventually consist of a crust of variable thickness, beneath which the normal epithelium is slowly spreading. If a few days later the crust be forcibly removed, either a small pocket of pus or a tough mass, adherent to the crust, will be found beneath, bordered by pink, new-formed epithelium which is growing in from the periphery. In some lesions the microscopic characters of the process are reflected in the appearances as presented to the naked eye to a greater extent than in others. Thus, in the vesicular ring two zones can at times be made out, an inner, opaque one and an outer, translucent zone. This agrees with the microscopic findings in some lesions where the leucocytes are seen beneath and to one side of the crust, while there is only clear serum in the peripheral part of the vesicle.

Departures from the type lesion were seen in certain of our animals. In some cases the vesicle ring does not completely form around the crust, so that at the height of the process the crust may be bordered in part by elevated and hyperæmic skin. In other lesions a vesicle may not at any time become recognizable to the naked eye. A variation which is not uncommon is for the lesion to undergo typical evolution, but for the different phases to be more or less delayed or accelerated. For example, a vesicular ring may be distinguished after 3 days or it may not be evident until after 6 days. In some lesions the process appears to start from a number of separate points instead of spreading symmetrically from the scratch.

The typical process is considerably modified by the character of the skin at the site of inoculation. Thus, on the thick and hairy skin of the back the several zones of the lesion, which are clear enough in an inoculation on the abdomen, are not distinguishable. The same phenomenon is seen when the skin of the tail is inoculated.

(*b*) *The occurrence and macroscopic appearance of the exanthem.*—In 65 monkeys inoculated on the skin of the abdomen with variola virus, in one form or another, a general exanthem was noted in 50 (77 per cent). In the animals in which vesicle contents was used for inoculation the exanthem occurred in 32 out of 40 (80 per cent). The exanthem was first noted on the seventh day of the disease in 7 animals, on the eighth day in 26, on the ninth day in 14, and on the tenth day in 3. When vesicle contents was used for the inoculation, the exanthem was first noted on the seventh day of the disease 5 times, on the eight day 18 times, on the ninth day 8 times, and on the tenth day once.

The extent of the exanthem varied greatly. In some animals only 1 typical lesion was present, while in others over 100 were found.

The distribution of the exanthem showed a partiality for certain regions. The face was most often the site of an eruption. Elsewhere, roughly in the order of frequency, it was present upon the wrists, the scrotum of the male, the region about the anus and base of the tail, on the palms of the hands and the soles of the feet, and on the inner aspect of the arms and thighs. The eruption seemed to avoid the trunk and the outer hairy surfaces of the limbs.

The evolution of the exanthem was rather constant. The first appearance of the eruption was as minute, pink papules, rarely exceeding 1 millimeter in diameter. On the next day this papule was larger, often measuring 2 millimeters and showing a vesicular structure. In the majority of cases the fluid contents of the lesion became cloudy on the next day, and the lesion was completely dried in another 24 hours. The exanthem,. therefore, has its complete evolution in about 4 days. In some animals the lesions pass through a longer period of development. In such lesions the papular, vesicular, and pustular stages could be recognized with as much certainty as in the primary lesions or as in the eruptive lesions of variola vera of man. The phenomenon was noted of the lesions appearing first on the face and later on other parts of the body and of their drying up in the order of their appearance.

The constitutional reaction, aside from the temperature, which might be taken as an indicator of the general reaction of the inoculated animal, showed little of a definite nature. At a time when the primary lesion is in its active stage, about the sixth to the eighth day of the disease, the animal sometimes shows some degree of anorexia and a tendency to droop, but at no time does it present such a constitutional reaction as is seen in the case of *variola vera*, of even moderate severity, in man.

The temperature reaction in *variola inoculata* in the monkey, differing from the same condition in its companion disease, vaccinia, presents a very definite curve. A comparison of the temperature charts of 20 animals, inoculated on the skin of the abdomen with vesicle contents, shows a marked rise in the body temperature on the sixth day of the disease in 14, on the seventh day of the disease in 2, and an indefinite one in 4. In only 3 of the animals was there no distinct elevation. This onset temperature may reach 41° C. In most cases the fever declines by lysis. The temperature reaction precedes the appearance of the exanthem by 24 to 48 hours.

The lymph nodes, which are interposed between the area of skin on which the primary lesion develops and the main lymph trunks, show a definite reaction. On the fourth or fifth day of the disease they are increased in size. This tumefaction increases and may, when the inoculation is on the abdomen, result in an enlargement of the individual nodes in the axilla to a diameter of 1 centimeter or more. At the time

of the greatest swelling the nodes are markedly tender. After the ninth or tenth day the nodes become smaller but remain firmer than normally for a considerable length of time.

The viscera of the animals killed during the disease showed no macroscopic lesions. Particular attention was paid to the bone marrow and testes, as these organs are the site of the only specific visceral lesion in *variola vera* in man.

(*c*) *Inoculations of the skin with variola disks.*—The inoculations in this series of animals resulted in a primary lesion which conformed to the type described above, save that the whole process was retarded in its evolution. However, the occurrence of the exanthem is in sharp contrast to that on the animals inoculated with the contents of the variola vesicle. The exanthem occurred in only 3 of the 5 animals, appearing in one on the eighth day, in the other 2 on the ninth day of the disease. In these 3 animals a total of only 7 eruptive lesions were found. The exanthem passed through its evolution rapidly and the individual lesions were small. The temperature reaction was like that in the preceding experiments in 3 of the animals, while in 2 it was indefinite. In other respects the results were similar to those in which the contents of the variola vesicle was used for inoculation.

(*d*) *Inoculation of the skin with dried pustule contents.*—In both animals of this series the primary lesion was typical in its development but somewhat delayed in its evolution. No exanthem was observed. The temperature reaction was the same as in the animals inoculated with the contents of the variola vesicle.

(*e*) *Inoculation of Macacus nemestrinus.*—In this monkey there is considerable variation from the type of the disease seen in *Macacus cynomologus*. This difference is principally shown in the evolution of the primary lesion. In this species the vesiculation of the lesion is much less definite, and there is an exaggeration of the œdema beneath it. The exanthem differed in no way from that observed in the Philippine monkey. The temperature reaction was less definite. In other respects the results of the inoculations were similar.

2. (*a*) *Histological examination of the primary lesions.*—The evolution of the lesion at the site of inoculation is characterized by a combination of degenerative, exudative, and reparative processes. Some one of these phenomena dominate the picture at different stages of the lesion, and in these various cell types figure.

The earlier lesions collected during the first, second, and third days of the disease primarily present the process of repair of a simple wound of the skin, but to this is added a change in the epithelial cells which border the incision. In these cells some degree of swelling and of degeneration is evident, although the usual reparative power of the *rete* is retained in sufficient degree to bridge the defect caused by the inoculation during the third day of the experiment. The cytoplasmic phases

of *Cytoryctes variolæ* are found in the cells of the *rete* during these early stages of the lesion.

From the third day of the disease onward the picture becomes more complicated. The degeneration of the epithelial cells is quite extensive and is evident for some distance from the line of inoculation. This degeneration is preceded by more or less swelling of the individual cells and some, though not wide spread, proliferation. The thickening of the epidermis about the inoculation is in the main due to the former process. Concomitant with the appearance of these phenomena we find fluid collected between the epithelial cells which finally leads to the formation of definite cavities, the vesicular space being partly contributed to by the solution of the swollen and degenerated epithelial cells. The polymorphonuclear leucocytes pass into the fluid of this vesicle, and their increasing numbers finally give to the lesion the macroscopic character which we designate as a pustule. While this vesiculation has been in progress, the crust which originated in the inspissated exudate in the inoculation scratch increases in extent and in thickness. The degeneration of the epithelial cells about the lesion is most marked where they form the floor of the vesicle, and becomes less and less as we pass to the peripheral portions of the lesion. Similar cell changes are found in the sheaths of the hair follicles close to the lesion.

While the vesicle has been forming in the epidermis, a reaction in the corium has become manifest. The first change, besides the collection of polymorphonuclear leucocytes in the vessels and their migration toward the vesicle, is enlargement and proliferation of the endothelial cells of the lymphatics and blood vessels. The connective-tissue cells in the corium undergo similar changes. Later in the disease a definite œdema of the corium and of the adjacent subcutaneous tissue is present, and an area of necrosis can be made out beneath the center of the lesion Associated with this œdema and necrosis there is a large increase in the number of endothelial cells. These cells appear not only in and around the lymph vessels and capillaries but are found in the surrounding tissue; they contain cytoplasmic phases of *Cytoryctes variolæ* both when in situ on the walls of capillaries and when free in the tissue. Mitoses in these cells are frequent and some are met with containing two nuclei. The phagocytic properties of these cells are shown by their including leucocytes and other cells. Lymphoid and plasma cells, together with a certain number of eosinophile leucocytes are found in the corium at this time.

During the later stages of the lesion the process of repair dominates the picture. The epithelium grows in from the sides and up from the hair sheaths and finally closes the defect caused by the variolous process. In the corium the solution of the necrotic tissue and the new formation of blood vessels and connective tissue shows repair to be active.

(*b*) *The exanthem.*—In the development of the exanthem, the process seen in the epithelium agrees with that found in the primary lesion.

However, in the corium the process of œdema and necrosis is lacking, but there is some degree of cell reaction evidenced by the enlargement and proliferation of endothelial cells. The emigration of polymorphonuclear leucocytes is a prominent feature of the process.

(*c*) The lymph nodes associated with the primary lesion show a marked reaction, consisting in proliferation of endothelial cells in the sinuses and in the follicles and in the active phagocytic properties of these cells. The presence of red blood corpuscles and of polymorphonuclear leucocytes in the sinuses and the small areas of hemorrhage in the follicles appear also to be a part of the process.

(*d*) The internal organs show nothing which can be interpreted as manifestations of the disease produced in the animal by the inoculation.

DISCUSSION.

We have seen that the inoculation of the skin of the monkey with variola virus brings about a disease which exhibits characters at once relating it to variola in man and to vaccinia in man and in animals. The disease which follows an inoculation of the skin of the abdomen of *Macacus cynomologus* with fresh variola virus consists essentially in—

(1) The development of a lesion at the site of inoculation.

(2) The appearance of a general cutaneous eruption of vesicular lesions.

(3) The enlargement of lymph nodes in the axillæ and groin.

(4) The constitutional reaction.

When we examine the primary lesion microscopically we find it to be a self-limiting process which passes through certain definite phases which are reflected in the gross appearances and are described as vesiculation, pustulation, and crusting.

When we turn to the lesions of the exanthem we find that the characteristic phases of the primary lesion are produced in them.

As we have said, the disease presents a series of characteristic phenomena, and when a number of animals are simultaneously inoculated we see that these phenomena bear a definite time relation to one another. If we emphasize this time element we find that the phenomena of the disease occur as follows.

The evolution of the primary lesion covers a period of about 14 days from the time of inoculation. The first portion of this period comprises the active evolution of the local process. This period of active growth terminates on about the seventh day of the disease. It is difficult exactly to say when the lesions stop developing, but after combining various observations, this date is selected as the probable average time for the acme of the active evolution of the primary lesion. During the remainder of the period the phenomena of repair are dominant in the lesion.

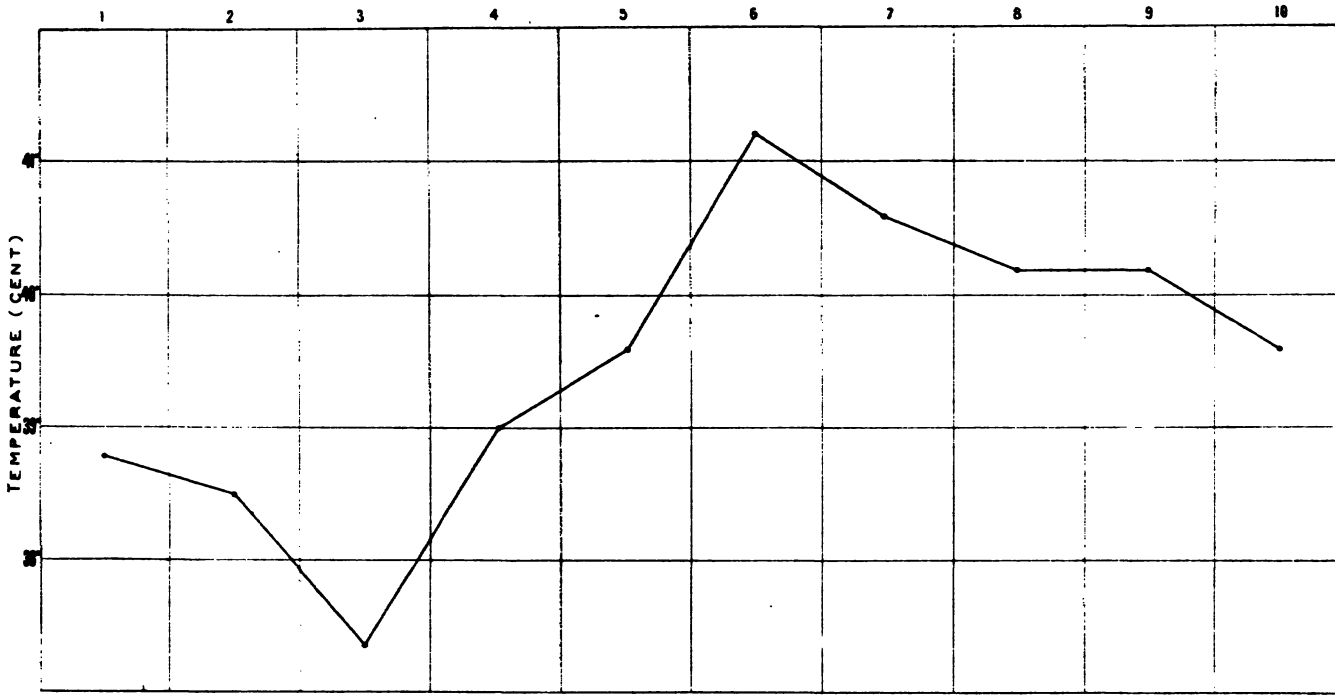
The general exanthem appears in the majority of cases on the eighth

day of the disease and has a duration of about 5 days, the first 2 of which are employed in growth and the last 2 in healing.

The lymph nodes show an enlargement on the fourth day of the disease and are always markedly enlarged on the fifth. They decrease in size during the crusting of the primary lesion, but they are firmer than normal for a considerable time after the healing of the process.

The constitutional reaction occurs at about the height of the active phase of the primary lesion—that is, on the sixth and seventh day of the disease. In a great majority of cases the temperature reaction begins on the sixth day of the disease and persists for 2 or 3 days. It is difficult to say just how long the fever lasts, as the decline is by lysis, but it is almost always within normal limits by the twelfth day.

FIG. 2. TEMPERATURE REACTION.



No. 118. VARIOLA INOCULATA IN THE MONKEY.

If we compare the disease produced in the monkey by cutaneous inoculation with variola virus with the disease which follows inoculation of that animal with vaccine virus we see at once that the processes are closely related. They are similar in that a self-limiting lesion appears at the site of inoculation, that the development of this lesion is associated with more or less constitutional reaction, and that certain lymph nodes become enlarged. The disease produced by inoculation with variola virus differs from vaccinia in that the primary lesion is usually followed by a general cutaneous eruption of lesions similar in many respects to the primary lesion and in that the temperature reaction is more abrupt in its onset and more intense.

If we compare the disease produced in the monkey by cutaneous inoculation with variola virus with the various manifestations of variola in man, we see that the disease produced is more like *variola inoculata* than any of the other forms. In fact the only differences which we find between the two lie in the time of occurrence of the general exanthem and in the duration of the temperature reaction. Thus, in the monkey we have the exanthem appearing on the eighth day of the disease, the temperature, which appeared on the sixth day, quickly falling by lysis, while in *variola inoculata* in man the exanthem appears on the eleventh and the temperature persists from the seventh to the ninth day. The development of the primary lesion and of the exanthem are practically the same in both.

When we compare *variola inoculata* in the monkey with *variola vera* in man we find that only certain characteristics of the disease type are held in common.

Different forms of virus, such as vesicle contents, pustule contents, and disk, were used for inoculations. On the skin, all these forms of virus produced typical primary lesions. However, it is to be noted that the nature of the contagium seemed to have an influence upon the occurrence and extent of the exanthem. We feel that these differences are explainable upon physical grounds and have to do with the reaction of the virus to external conditions rather than to any difference in the virus which is inherent to different ages of the lesion from which it is collected. This question will be taken up in another section of this article.

In the course of our experiments monkeys of all ages and of both sexes were employed. We did not observe any difference in the reactions of these animals which could be attributed to these factors. The general physical condition of the animal did not seem to affect the results of the inoculations.

The histological study of the specific lesions and of the viscera in *variola inoculata* in the monkey adds some details to our picture of the disease. We see that the specific lesions are similar in most respects to the vaccine lesions of man and of animals, and to the lesions of the

exanthem of *variola vera* in man. As has been pointed out,[6] the specific lesions of *variola inoculata* in the monkey differ from the lesions of the exanthem in *variola vera* in man in the greater prominence of the polynuclear leucocytes in the former. The primary lesions of *variola inoculata* in the monkey differ from the vaccine lesions of that animal in the extent and character of the process in the corium beneath the lesion, it being more intense in *variola inoculata.*

It is evident that the lymph spaces of the skin are flooded with virus at the time of the inoculation, and this fact may be a factor in the early development of the exanthem in the inoculated disease. From the fact that cytoryctes are demonstrable in the endothelial cells of the capillaries in the corium beneath a primary lesion of five days' duration, it seems probable that at the time of inoculation, cells of this type become infected. Such infected cells in the lymph spaces or in the capillaries might easily be swept away in the circulation, and lodging in skin capillaries become the focus for an exanthem.

The absence of focal lesions in the bone marrow and testicles in *variola inoculata* in the monkey emphasizes the difference already mentioned between the disease experimentally produced in the monkey and *variola vera* in man.

CONCLUSIONS.

(1) Inoculation of the skin of the monkey (*M. cynomologus* and *M. nemestrinus*) with variola virus produces a disease in which all the essential characteristics are identical with those of *variola inoculata* in man.

(2) *Variola inoculata* in the monkey differs from *variola inoculata* in man in that the fever has a shorter duration and the exanthem appears at an earlier date.

(3) *Variola inoculata* in the monkey is as distinct a clinical entity as is *variola inoculata* in man.

(4) *Cytoryctes variolæ* are found in the endothelial cells of the capillaries in the corium beneath the primary lesion of *variola inoculata.*

2. VARIOLA INOCULATA IN THE ORANG-UTAN.

Introduction.—The experiments here reported were performed to determine the reaction of the orang-utan to inoculation with variola virus and to obtain material for the microscopic study of the specific lesions and of the morphology of the causitive organism in this species of animal. Four orang-utans were procured for this work, but unfortunately only two of them survived long enough for the experiments to be carried out. These experiments are the first in which anthrapoid apes have been used as the experimental animal in a study of small pox. The systematic position of the orang-utan in the animal kingdom makes it of peculiar

[6] Magrath and Brinckerhoff: *Jour. Med. Research,* **11**, 230.

value in the comparative study of such a disease, it being susceptible both to diseases peculiar to animals (hæmorrhagic septicæmia) and to those common to man and to animals (amoebic dysentery). This makes the animal an ideal one for bridging the gap between the monkey and man in the study of the reactions of various mammalian hosts to a given disease-producing parasite. We regret that owing to the difficulty of acclimating these animals our data are not as abundant as might be desired.

Technique.—Two young female orang-utans were inoculated on the skin of the abdomen with variola virus in the same way as were the monkeys in the work the details of which have already been described. The animals were observed daily and the evolution of the lesion at the site of the inoculation and the constitutional reaction was recorded. Material for the histological examination of the specific lesions was collected. The details of the experiments are as follows:

No. 197. Young female orang-utan. The animal was first exposed to smallpox fomites. The results of this experiment will be considered in another article.

Twenty-one days after the exposure, the animal was inoculated in 12 places on the abdomen with virus No. 252. Forty-eight hours after inoculation there was slight elevation about the scratches. The deep pigmentation of the skin made it impossible to tell whether or not hyperæmia was present.

Five days. Along each line of inoculation there was a narrow, yellow crust seated upon a vague elevation. On gentle pressure turbid fluid exuded from beneath the crust. A small amount of this fluid was used to inoculate a Philippine monkey on the skin of the abdomen. This animal developed a typical pock at the site of inoculation and subsequently a profuse general exanthem.

Six days. The primary lesions were somewhat more prominent and the whole of the area beneath them was indurated. Axillary lymph nodes enlarged, firm and tender.

Seven days. The animal had scratched the lesions and they presented shallow ulcerations with ragged and often bleeding edges. The subcutaneous œdema had increased in extent, causing a brawny induration over the whole field of inoculation. The animal died during the night of an intercurrent infection.

No. 198. Young female orang-utan. Inoculated in 12 places on the skin of the abdomen with virus No. 199. After 24 hours slight elevation was apparent along the lines of inoculation. Body temperature 36°.1 C.

Four days. A narrow crust marked the scratch and was bordered by a distinct elevation. No change of color could be distinguished on account of the deep pigmentation of the skin. Body temperature 35°.9 C.

Five days. The elevation of the lesions had increased and they had become circumscribed. Body temperature 36°.6 C.

Six days. The zone of elevation was distinctly vesicular near the crust and clear serum oozed out on gentle pressure. Body temperature 36° C.

Seven days. Lesions had increased somewhat in extent and the crust had spread. Body temperature 35°.7 C.

Eight days. Animal found dead in cage early in the morning. *Rigor mortis* present, body still warm. Autopsy at once. *Skin.*—The inoculation sites present crusts, about which is a shallow cavity filled with turbid fluid. This vesicle appears to lie between the true skin and the epidermis. The border of the lesion shows some thickening of the skin, but this is not nearly so marked as at a corre-

sponding period in the primary lesion of *variola inoculata* in the monkey. No evidence of a general exanthem is present. Axillary lymph nodes enlarged, measuring 1 to 1.5 centimeters in diameter, deep-red in color, and on section rather dry. Peritoneal cavity contains one liter of clear, straw-colored fluid. Surfaces of normal color and texture. Pleural and pericardial cavities normal. *Heart.*—Valves and cavities normal; myocardium red-brown in color and of firm consistency. *Lungs.*—Normal. *Spleen.*—Capsule smooth, purple, on section pulp rather dry, color deep red-brown. Malpighian bodies and trabeculæ not prominent. *Liver.*—General surface smooth and yellow-brown in color. On section, markings distinct and consistency normal. *Pancreas.*—Normal. *Gastro-enteric tract.*—Stomach normal. The mucosa of the cæcum and of the colon presents numerous punctate hemorrhages. *Kidneys.*—Capsule strips readily from a smooth yellow-brown surface. On section general color opaque yellowish-brown with irregular areas of injection in the cortex. Glomeruli visible as bright, red points. Genital organs and bladder normal. Bone marrow of femur deep red, homogeneous, and of firm consistency. Brain and meninges normal. Smears from the heart's blood show immense numbers of small, short, bacilli, which take a polar stain with Loeffler's methylene blue.

Bacteriological examination. Cultures from the heart's blood, the liver, and the spleen yield an organism which was identified by Dr. W. B. Wherry, bacteriologist of the Biological Laboratory, Bureau of Science, as belonging to the group of organisms causing hemorrhagic septicæmia in animals.

Histological examination. Primary lesion. Six days. The point of inoculation is marked by a complete destruction of the epithelium. The material present here consists of a lamella of cornified epithelium which fuses with a more or less homogeneous crust in which cell elements can occasionally be recognized. The upper layers of the corium at this place are more or less extensively necrosed, the connective-tissue fibers are swollen and fused with one another and with the crust. Cell detritus is scattered through this tissue. A fibrin network lies in the lacunæ of the corium in this region. On either side of the line of inoculation, immediately adjoining the crust, the cells of the *rete* are recognizable but are very much degenerated. Between these degenerated cells and the cornified layer are finely granular areas of the size of epithelial cells, each outlined by a membrane, but not containing recognizable cell structure. Polymorphonuclear leucocytes are present in this tissue, their nuclei often fragmented or show other signs of degeneration. As we pass outward from the line of inoculation the degenerated *rete* splits horizontally, one layer more or less imperfect following along the surface of the corium, while the other, still merging superficially with the degenerated strata above, curves up to form an almost complete band across the vesicle. Between this superficial layer of the *rete* and the corium is a lenticular cavity filled with a fine, granular material and containing a fibrin network. At the outer limits of the vesicle the cornified layer sweeps downward to merge with the thickened epidermis which marks the outer limits of the lesion. The layer of *rete* which forms the floor of the vesicle likewise runs into this thickened region. Beyond the main vesicle small cavities are to be seen in the middle layers of the epidermis. Some of these are evidently formed by hydropic degeneration of the cells, the cell membranes persisting as partitions across the cavity. In others these partitions have disappeared and the process seems in part due to an accumulation of fluid within and between these cell cavities. On the other side of the line of inoculation vesicle formation is evident for a considerable distance. Here, however, the *rete* remains intact and the collection of fluid is between and within the cells of the *stratum granulosum* and *stratum spinosum*. In places this vesicle is traversed by more or less vertical partitions composed of compressed epithelial cells.

The reaction of the corium is evidenced by some infiltration, with polymorphonuclear leucocytes and a certain degree of swelling of the endothelial cells lining the blood vessels and lymphatics of the corium adjacent to the site of the inoculation. Immediately beneath the lesion endothelial cells of lymphatics and of capillaries were found which contained in their protoplasm cytoplasmic phases of *Cytoryctes variolæ*.

The epithelial cells of the lesions contain many stages of the parasite. Both the cytoplasmic and the nuclear phases were well represented.

Seven and eight days. Lesions of these durations showed the same characteristics as did that described above. The vesicle becomes more extensive and the necrotic area beneath the crust larger. The coalescence of the lateral vesicles often left single cells or islands of cells in a fair state of preservation, which were to be found in various stages of agglomeration on the way to the formation of trabeculæ, or partitions in the large vesicle.

Viscera. Histological study of the internal organs did not reveal any lesions of a variolous nature. All through the organs, wherever blood vessels were cut, short bacilli having the morphology and staining peculiarities of the bacillus of hemorrhagic septicæmia were readily demonstrable.

SUMMARY.

The evolution of the primary lesion at the site of inoculation with variola virus in the orang-utan is similar to that which follows the inoculation in the monkey and in man. The thickness and the deep pigmentation of the skin of this animal rendered the appearance less characteristic to the naked eye than in *Macacus cynomologus*. The death of the animals, of intercurrent disease, before a general exanthem might be expected to develop, deprived us of data upon this point. Both animals showed a marked constitutional reaction, but this, because of the existence of an intercurrent disease, can not be interpreted as resulting from the smallpox process.

The histological study of the primary lesions and of the viscera of the orang-utan inoculated with variola virus shows the process to be essentially similar to that which follows inoculation of the monkey with the same virus. There seems to be some difference in the degree of reaction in the corium beneath the primary lesion, it being notably less in the orang-utan. The histogenesis of the cutaneous vesicle is similar and we note the absence, as in the monkey, of focal lesions in the bone marrow. The most striking thing about the primary lesions seems to be their richness in nuclear forms of cytoryctes. This matter will be taken up in detail in another paper and so will not here be further commented upon.

CONCLUSIONS.

(1) The orang-utan is susceptible to *variola inoculata*.

(2) The evolution of the specific lesion at the site of inoculation is comparable with that which follows similar inoculations in the monkey (*M. cynomologus* and *M. nemestrinus*).

(3) The primary lesions of *variola inoculata* in the orang-utan stand closer to the cutaneous lesions of *variola vera* in man than do the primary

cutaneous lesions of *variola inoculata* in the monkey in respect to richness in forms of *Cytoryctes variolæ*, and particularly in the number of nuclear forms present.

3. VARIOLOUS KERATITIS IN MACACUS CYNOMOLOGUS.

Introduction.—In this section will be considered the results of a series of inoculations of the cornea of the Philippine monkey with variola virus. These experiments were undertaken to determine what variations in the type of disease might result from a change in the locus of inoculation; they also yielded material for the histological examination of the specific lesion in tissue which is ideal for the study of cell changes owing to perfect preservation and the relative simplicity of the tissue elements.

Technique.—The methods of inoculation and of observation were the same as those described in the section upon vaccinal keratitis in the monkey.

Details of experiments.—Eighteen animals were employed in this series, of which the following experiments are selected to be given in detail:

No. 223. Monkey inoculated on both corneas with virus No. 200. After 48 hours slight unevenness was apparent along the line of inoculation. After 96 hours a minute defect in the corneal epithelium was present in the inoculated area. Chloroformed after 8 days. The cornea presents a small defect in the epithelium with very slight unevenness about it. No photophobia was observed. There was no general exanthem.

Histological examination showed proliferation of the epithelium about the inoculated wound and the presence of cytoryctes.

No. 224. Monkey was inoculated on the cornea with virus No. 200. After 48 hours the cornea was slightly uneven along the line of inoculation. Photophobia and haziness of the cornea was observed after 96 hours. Conjunctivitis was not present. On the sixth day of the disease a general exanthem consisting of 5 small vesicles was observed on the face and extremities. On the next day 5 new vesicles appeared. The animal was choloroformed on the seventh day. At autopsy no evidence of an initial lesion other than that on the cornea was demonstrable.

Histological examination. At the site of inoculation enormous numbers of cytoryctes were present in the epithelial cells. No leucocytes were found in the lesion or in the corneal substance about it.

SUMMARY.

The inoculation of the cornea with vesicle contents was followed by the development of a lesion which had much in common with that which follows a similar inoculation on the rabbit. The following summary of the macroscopic appearances is based upon the observation of 18 experiments similar to those given above.

After 24 hours there is some roughening of the surface along the line of inoculation. After 48 hours there follows more or less loss of substance at the site of the inoculation incision. This loss of substance is

not so great as in vaccination of the cornea. When pyogenic infection does not complicate the process there is no opacity of the cornea or conjunctivitis, and the lesions heal after a variable period. In 2 animals, which were allowed to survive long enough for the eruption to appear, an exanthem was seen on the sixth and seventh days of the disease. The evolution and extent of the exanthem was like that following the skin inoculation. Another animal, kept under observation for a long period, did not develop an exanthem.

Histological examination shows that the process at the site of inoculation with variola virus on the cornea of the monkey consists primarily in degeneration and in proliferation of the epithelial cells. Seventy-two hours after the inoculation when the process is at its height the line of inoculation is marked by a defect in the epithelium, below which there may be a slight destruction of the corneal substance. The epithelial cells about this defect may be swollen and separated one from another, and show various degrees of degeneration. As we pass from the center of the lesion toward the periphery we find the epithelium much thickened. This increase in the thickness is in part due to swelling of the individual cells, shown particularly by those of the lower layer, which appear pale and assume a cuboidal or cylindrical form, and in part to an increase in the number of cells. At the point of greatest thickening the epithelium may measure twice its normal depth. In lesions of greater duration the degeneration of the individual cells and collection of fluid between the cells may occasionally result in the formation of minute cavities in the thickened epithelium, which are analogous to the vesicle of the specific skin lesion.

Polynuclear leucocytes do not form a prominent feature of the corneal lesions. In many sections a prolonged search is necessary to find a single cell of this type. When the inoculation wound penetrates the corneal substance polynuclear leucocytes are more numerous. The paucity of these cells in the lesion is in strong contrast with the condition in the cutaneous smallpox lesions in the monkey.

Cytoryctes variolæ were present in all the lesions examined. Their morphology and staining reactions were identical with those found in the skin lesions. No nuclear phases were present. The parasites were found as early as 18 hours after the inoculation and persisted through the eleventh day, that date being the last on which a microscopic examination was made.

DISCUSSION.

When we compare the lesion produced on the cornea of the monkey by inoculation with variola virus with vaccinial keratitis in the same animal we see at once that we have to do with a similar process. The only striking difference which these two lesions present is that in the variolous keratitis there is less exudation and the epithelium of the lesion does not become detached and cause a large superficial defect as in the

vaccinial lesion. In this respect variolous keratitis in the monkey approaches more nearly to vaccinial or variolous keratitis in the rabbit than does vaccinial keratitis in the monkey.

The small part played by the polynuclear leucocyte in the variolous keratitis contrasts strongly with the prominence of this cell in the specific cutaneous lesions of the disease in the monkey. It seems probable that in the skin inoculation the presence of a large number of these cells is conditioned by the destruction of tissue incident to the inoculation and that they continue to be attracted, not so much by the variola organism or its products as by the presence of substances set free in the process of cell destruction incident to the activity of the parasite and the other organisms which gain access to the lesion. As all such degeneration products are retained at the site of inoculation by the crust, in the case of a cutaneous lesion, while they readily escape in the case of the corneal one, it is easy to understand why in the former case large numbers of leucocytes pass from the vessels to the lesion.

The absence of nuclear phases of *cytoryctes variolæ* in the corneal lesion was disappointing. It is possible that there is some inherent difference in the cells of the cornea which makes them unsuitable for the development of the parasite beyond the cytoplasmic stage, though we are inclined to regard the absence of nuclear forms in these lesions as being due to the action of physical factors. In a typical corneal lesion the cells are probably cast off from the surface before the nuclear forms are produced. It is possible that oblique incisions into the corneal substance, in which islands of epithelial cells would be retained, might show a development of the nuclear forms of the parasite.

In a previous section we have shown that the endothelial cells of capillaries beneath the primary skin lesions of *variola inoculata* are invaded by cytoryctes. From this we have been led to believe that some such process is involved in the dissemination of the organisms to form the exanthem. From the considerations of the time elements it seems probable that such infected endothelial cells are set free at an earlier stage in the disease than the demonstration of the infected endothelial cells beneath the primary lesion. The fact that an exanthem follows the development of a lesion in the nonvascular cornea, where extension of the process to blood vessels does not occur, suggests that the distribution of the organism, if brought about through the intermediation of endothelial cells, is due to an infection of the cells which line lymph channels.

CONCLUSIONS.

(1) Inoculation of the cornea of the monkey (*M. cynomologus*) with variola virus produces a specific lesion characterized by swelling, proliferation, and varying degrees of degeneration of the epithelial cells.

(2) The lesion is similar to that produced by inoculation of the cornea of the rabbit with vaccine or with variola virus.

(3) The lesion results in less destruction of the corneal epithelium than follows similar inoculations of the cornea of the monkey with vaccine virus.

(4) The lesion on the cornea differs from the variolous lesion on the skin of the monkey in that exudation does not play as prominent a part and that true vesicle formation does not occur.

(5) *Cytoryctes variolæ* are present in the lesion up to 11 days after the inoculation, but nuclear forms of the parasite are not found.

(6) A variolous lesion on the cornea of the monkey may be followed by a general exanthem which appears on the same day as after skin inoculation. We therefore identify the disease produced in the monkey by variolation on the cornea as *variola inoculata.*

4. VARIOLA INOCULATA FOLLOWING INOCULATION OF THE MUCOUS MEMBRANE OF THE MONKEY.

Introduction.—In the preceding sections of this paper we have detailed the results which follow the inoculation of the skin and the cornea of the monkey with variola virus. We will present here a series of experiments which show the results of inoculation of that animal upon the mucous membrane of the nose, the lip, and the palate. The experiments also yielded material for the study of the variola organism and the histology of the specific lesion on the mucous membrane.

Technique.—The method of inoculation and of observation was the same as that followed in the series of inoculations of the mucous membranes of the monkey with vaccine virus.

Clinical course of the disease.—Twenty-nine animals were used in this series of inoculations, of which the following are selected to be described in detail:

No. 125. Adult male, *Macacus cynomologus.* Inoculated on the left side of the nasal septum, on the inner side of the lower lip, and on the left palate with variola virus No. 167 (vesicle contents). Body temperature 40° C.

Twenty-four hours after inoculation a slight elevation is noted on the nasal septum. The lip and palate are negative. Body temperature 38°.8 C.

Forty-eight hours. On the lip a narrow white line surrounded by hyperæmic mucous membrane marks the site of inoculation. Nose and palate negative. Body temperature 40° C.

Three days. The nose shows considerable swelling of the septum, but no distinct lesion can be distinguished. The lip presents a white area, 1 × 3 millimeters, which is slightly elevated and has a translucent appearance. The palate shows a small, gray spot on the line of inoculation. Body temperature 40° C.

Four days. Nose negative. The lip shows a white, opaque area 1 × 3 millimeters, with a ragged elevated edge, about which the mucosa is distinctly reddened. The palate presents a white, slightly elevated area, 2 × 4 millimeters, the surface of which is unbroken. Body temperature 40° C.

Five days. Nose negative. The lip shows an opaque, white area, covered in part by the remains of the macerated epithelium, and surrounded by a dull pink elevated border. The palate presents an elevated area 2 × 4 millimeters in extent and of a gray-white color. About this are smaller similar spots. Body temperature 39°.5 C.

Six days. The nose presents an opaque area on the septum surrounded by a bright red areola. The lip is much swollen and the lesion shows an area of erosion surrounded by a ragged edge of elevated and hyperæmic mucous membrane. The lesions on the palate have increased somewhat in size and show a distinct red areola. Body temperature 40°.2 C.

Seven days. Considerable mucopurulent discharge from the nostril; swelling of the mucous membrane prevents inspection. Lesion on lip as before. The palate shows some increase in the size of the lesion. Body temperature 40° C.

Eight days. The swelling of the mucous membrane in the nose continues. On the lip the lesion presents as an ulceration with marked inflammatory reaction about it. On the palate the lesion shows no erosion and has ceased to spread. Body temperature 40°.4 C.

Nine days. Lesions as described yesterday. A general exanthem was carefully searched for but not found. Animal killed and autopsy done at once. No macroscopic evidence of disease except at the sites of inoculation.

No. 126. Half-grown male, *Macacus cynomologus.* Inoculated in the same way and with the same virus as the preceding animal. Body temperature 39° C.

After 24 hours the nose shows a delicate crust on a slightly elevated area of mucous membrane. On the lip a small lacerated wound marks the point of inoculation. The palate presents some reddening of the mucosa about the scratch. Body temperature 38°. C.

Forty-eight hours. Lesions as above described. Body temperature 39° C.

Three days. In the nose the elevation about the crust has increased. The lip shows an elevated, opaque, white area, 3 × 4 millimeters, surrounded by a diffuse red flush. On the palate is a slightly elevated grayish area, 2 × 4 millimeters in extent, surrounded by hyperæmic mucosa. Body temperature 40°.4 C.

Four days. Nose as before, save that the elevation has taken on a gray tint. The lip presents a macerated appearance over the area occupied by the lesion. The lesion on the palate has increasd in size. Body temperature 40°.5 C.

Five days. On the palate the lesion is eroded, otherwise there is no change. Body temperature 39°.5 C.

Six days. The nose presents a narrow crust on a pink elevation. The lesion on the lip shows signs of healing. Palate as before. The top of the tongue near its tip presents a gray elevated area, 2 millimeters across, surrounded by a pink areola. Lesions show no change since yesterday. Animal killed and autopsy at once made. Viscera show no macroscopic lesions. A single small vesicle outside the left eye is shown to be specific by microscopic examination. No other exanthem found.

No. 127. Young female, *Macacus cynomologus.* Inoculated in the same way and with the same virus as the preceding monkeys. Body temperature 39°.2 C.

Twenty-four hours after. Slight elevation and reddening at the points of inoculation. Body temperature 38° C.

Forty-eight hours. In the nose there is reddening of the mucosa on the septum. On the lip a yellow line surrounded by slightly elevated and pink mucosa marks the site of inoculation. Palate negative. Body temperature 39° C.

Three days. Nose negative. Lip shows a gray line on a white elevated area 2.5 × 5 millimeters, the edge of which is translucent. Palate presents a grayish-white line 3 millimeters long. Body temperature 39°.5 C.

Four days. Nose negative. The lip shows a gray-white area, 2 × 5 millimeters, with a pink edge and a granular surface. Palate as before. Body temperature 39°.5 C.

Five days. Nose negative. The lip is swollen and presents an erosion with a yellow surface and a thickened, pink edge 2 × 4 millimeters in extent. Palate

presents an eroded area 2 × 5 millimeters, with a slightly depressed gray base and an elevated margin which is surrounded by reddened mucosa. Body temperature 38°.5 C.

Six days. Lesions as before but somewhat increased in size. Body temperature 39°.5 C.

Seven days. Nose presents a narrow crust about the orifice of the left nostril. The lip shows a large erosion 1 centimeter across, the base of which is covered by yellow, macerated material and is surrounded by an elevated, ragged, pink border. Lesion on palate has increased in size. Body temperature 38°.8 C.

Eight days. Nose shows an eroded area extending from the septum out upon the surrounding skin. Lesion on lip as before. Palate presents a long, narrow ulceration 2 × 8 millimeters in extent, with a white, elevated edge, and surrounded by a red areola. One small, pink, papular elevation on the inner side of each thigh and on right arm. Histological examination shows this lesion to be variolous. Body temperature 38°.5 C.

Animal killed; at autopsy organs appear normal.

No. 128. Full grown female, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding monkeys. Body temperature 39° C.

After 24 hours there is slight reddening of the mucosa about the scratches. Body temperature 38°.2 C.

Forty-eight hours. Lesions as before. Body temperature 38°.2 C.

Three days. The lip is swollen and presents a white area, 1 by 3 millimeters, slightly elevated and surrounded by a pink areola. Palate presents 2 yellow elevations 2 by 4 millimeters in extent with a red periphery. Body temperature 39°.5 C.

Four days. Lesion on lip is excoriated. Palate as before, but lesion has spread somewhat. Body temperature 39° C.

Five days. Lip shows an eroded area with a gray base and a white, elevated edge, surrounded by reddened mucous membrane. The whole lower lip distinctly swollen and indurated. Palate as before. Body temperature 38°.2 C.

Six days. Swelling of the lip more marked. The base of the lesion is yellow and the edge ragged. A minute, pink papular elevation appears on the gum opposite the lesion. Probably an auto-inoculation. Palate as before. Body temperature 39° C.

Seven days. Lesions as described but somewhat increased in extent. Body temperature 38°.5 C.

Eight days. From this time on the primary lesions healed without complication. A general exanthem was not found either before or after this date. Body temperature 37°.5 C.

No. 129. Full grown male, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding animals. Body temperature 38°.5 C.

Twenty-four hours after inoculation the lip and palate show a white line surrounded by hyperæmic mucosa. Nose negative. Body temperature 39°.8 C.

Forty-eight hours. Lip and palate show irregular elevation with opacity and and a peripheral flush at the site of inoculation. Nose negative. Body temperature 38°.2 C.

Three days. Lip presents a yellow, eroded area, 2 by 5 millimeters, surrounded by a pink areola. Palate shows an opaque, white area, 2 by 5 millimeters, surrounded by reddened mucosa. Body temperature 39° C.

Five days. Lesions on lip and palate somewhat increased in extent. The inoculation in the nose evidently did not take. Body temperature 39°.4 C. Animal killed and autopsied at once. Viscera, present no macroscopic lesions.

A series of 10 monkeys were inoculated on the palate alone and a series of 9 upon the nasal mucosa alone. The results of the inoculations were similar to the experiments detailed above.

SUMMARY.

The lesion which develops on the inner side of the lip of the monkey following inoculation with vesicle contents presents the following macroscopic characters.

After 24 hours the site of inoculation shows, at most, a slight reddening of the mucous membrane about the scratch.

After 2 days a narrow, white line is seen, which is surrounded by a faint, red flush.

After 3 days there is a definite, opaque, white, area, 2 or more millimeters in extent, slightly elevated above the general surface. This area is more or less eroded and is surrounded by a distinct zone of hyperæmic mucous membrane.

After 4 days the opaque area is somewhat eroded and presents a shallow ulcer with an elevated, white, often sinous, edge, which is bordered externally by reddened mucosa.

From this time on the lesion presents the same characteristics, the only change being due to a gradual extension of the process. After 8 or 9 days the peripheral flush fades and healing begins. This process results in complete repair after about a week.

Inoculation of the palate causes a lesion similar to that following inoculation of the lip, and the lesion runs essentially the same course. In this situation the lesion is less apt to become eroded.

After inoculation of the mucous membrane of the nose it is difficult to follow the process from day to day, as at the time when the lesion is undergoing its active evolution the swelling of the mucous membrane which accompanies the process prevents inspection during life. From study of the site of inoculation in animals killed at various periods it is seen that the evolution of the lesion in the nose differs principally from those on the lip and palate in that there is less tendency to form an ulcer. When the inoculation is near the anterior nares the process tends to spread out on the skin about the nostril and then takes on the characteristics of a skin inoculation.

No notable constitutional reaction followed the inoculation of the mucous membrane. The temperature reaction was indefinite. A general exanthem was observed in 2 of the monkeys.

Histology of the primary lesion.—Lip: Sections from lesions collected 25 and 53 hours after inoculation show no evidence of a specific process. The defect in the epithelium caused by the inoculation has completely been repaired, and the only evidence of the wound is a small collection of fibrin and polynuclear leucocytes just beneath the epithelium.

Three days after inoculation the lesion shows specific characters and we find an area in which the epithelium presents a pathological change, and beneath this the tissue is infiltrated with polynuclear leucocytes. The changes in the epithelium consist in degeneration and disintegration of the epithelial cells, together with more or less accumulation of fluid in and between the cells. This accumulation of fluid occurs not only in the degenerating portion of the epithelium but also at the sides of the lesion. This process is similar to that seen in a skin inoculation of the same duration.

The degeneration of the epithelium is not uniform, and we find islands of comparatively normal cells in the midst of areas where the affinity of the nuclei of the epithelial cell for basic stains is lost. Polynuclear leucocytes are present in large numbers. The epithelium at the edge of the lesion is somewhat thickened, apparently as a result of the swelling of individual cells.

After 4 days the lesion shows the same characteristics, save that there is considerable loss of substance in the area of degenerated epithelium and the lesion is more extensive. At this time the reaction in the tissue beneath the lesion is well marked and proliferation and enlargement of the endothelial cells of the lymphatics and blood vessels is apparent. Many elements of the lymphoid and plasma cell series are present about the vessels beneath the lesion.

Five and 6 days after inoculation the lesions are similar in character to those just described, but the necrotic area becomes sharply limited and the inflammatory reaction beneath is more intense.

Lesions of 7 days' duration show evidence of beginning repair. The epithelium at the edge of the lesion is normal and the lesions consist of a sharply circumscribed ulceration, in the depths of which repair is active. Later lesions show the epithelium growing inward to close the defect, and new formed blood vessels and young connective-tissue cells are much in evidence in the tissue beneath.

In the lesions of 3, 4, 5, 6, and 7 days' duration epithelial cells containing cytoplasmic phases of *Cytoryctes variolæ* are of frequent occurrence. The earlier forms of the parasite occur at the margin of the lesion, the later ones nearer the center. Nuclear phases of the parasite are also found, but they occur later than the cytoplasmic phases.

Nose.—The primary lesions in the nose vary in character according to the locus of inoculation. When the incision is near the orifice of the nostril, on a stratified epithelium, the lesion conforms to the type described on the lip. When the lesion is higher up, upon a columnar epithelium, the process in the submucous tissue is most marked. In such a situation the bulk of the degenerated epithelial cells seem to be carried off almost at once, and we find but little thickening of the mucous layer. However, the submucous tissue shows accumulations of lymphoid and plasma cells, enlargement and proliferation of the endothelial cells of the

lymphatics and blood vessels, and a marked polynuclear leucocyte infiltration.

Both cytoplasmic and nuclear phases of *cytoryctes variolæ* are present in the epithelial cells. In one lesion, of 5 days' duration, an endothelial cell was found on the wall of a lymphatic, just beneath the epithelium, which contained a cytoplasmic form of the parasite.

Palate.—The histology of the primary lesion in this situation is similar to that seen in the lesion on the lip. A lymphatic with cytoplasmic forms of cytoryctes in the endothelial cells lining its wall was found beneath a lesion of 5 days' duration.

Histological examination of the viscera showed no lesions. The bone marrow and testicles were carefully examined for focal lesions, such as are found in these organs in *variola vera* in man, but none could be demonstrated.

DISCUSSION.

The disease produced by variolation of the monkey upon the mucous membrane of the lip, nose, and palate is characterized by the development of a self-limiting lesion at the site of inoculation, which may be followed by a general cutaneous exanthem and be associated with an indefinite constitutional reaction.

If we compare the initial lesion produced on the mucous membrane with that which follows a similar inoculation on the skin we see that the two processes are similar, in that they run a definite course and tend to heal after about the same interval of time. The microscopic study of these lesions shows them to be the result of similar cell changes, and in each the parasite, *cytoryctes variolæ*, is found associated with the process. The lesions differ in that on the mucous membrane the absence of crust prevents the development of a vesicle or pustule, although an accumulation of fluid between the cells is in evidence at certain stages of the lesion. The primary lesion on the mucous membrane, of 4 or 5 days' duration, simulates closely a skin lesion of the same duration which has lost its crust through rough manipulation. The primary lesion on the mucous membrane also differs from that upon the skin in that the process in the tissue beneath the lesion is more exudative and proliferative than necrotic.

When we compare the other manifestations of the disease following variolation of the mucous membrane with that following skin inoculation a decided difference is found. We see that a general exanthem is much less apt to follow the inoculation of the mucous membrane. Of 19 animals inoculated in the nose, on the lip, or on the palate only 2 showed a general exanthem. In both these animals the lesions of the eruption were few in number and required microscopic study for their positive diagnosis. The exanthem occurred in a trifle over 10 per cent of the animals. This is in sharp contrast with the occurrence of the exanthem

in *variola inoculata* from skin inoculation, where an eruption develops in from 70 to 80 per cent of the animals.

The constitutional reaction in monkeys variolated upon the mucous membrane differs in degree from that in those variolated upon the skin. We do not find such an abrupt elevation in the body temperature, and malaise and anorexia are absent. It seems reasonable to suppose that this relatively slight constitutional reaction is conditioned by the physical conditions at the *locus* of inoculation. The initial lesion on the mucous membrane is practically an open wound from the beginning, and consequently the products of cell destruction and any toxins produced by the specific organism can readily escape. The systemic absorption from lesions on the mucous membrane must be quantitatively much less than from the lesions upon the skin.

We are inclined to attribute the infrequency of the exanthem in this series of animals to physical conditions at the *locus* of the primary lesion. At the time of inoculation the amount of virus which enters and remains in the scratch must be notably less than when the skin is inoculated, owing to the fact that the surface inoculated is bathed with fluids and any exudation stream set up by the trauma of the inoculation would be assisted by the moist condition of the surface and the action of similar opposed surfaces to carry off the bulk of the virus. In these inoculations there can be no such flooding of the lymphatics about the scratch as must occur after similar inoculations of the skin.

The type of disease produced by variolation upon the mucous membrane conforms in general to that which follows inoculation of the skin. The nature of the primary lesion and the time of occurrence of the exanthem relate the disease at once to *variola inoculata*. The absence of focal lesions in the bone marrow and testicle differentiate the disease from *variola vera* in man.

CONCLUSIONS.

(1) Inoculation of the mucous membrane of the lip, the nose, or the palate of the monkey (*M. cynomologus*) with variola virus produces a disease which conforms to the type of *variola inoculata*.

(2) The primary lesion on the mucous membrane is similar, cytologically and histologically, to that which follows variolation of the skin.

(3) *Cytoryctes variolæ*, in both the protoplasmic and the nuclear phases, are present in the lesions.

(4) Cytoplasmic forms of the parasite are found invading endothelial cells of lymphatics beneath the lesions of 5 days' duration.

5. ON THE OCCURRENCE OF VARIOLA VERA IN MONKEYS AND IN THE ORANG-UTAN.

In the preceding sections we have shown that the monkey and the orang-utan react in a definite manner to inoculation with the virus of smallpox. This reaction consists in the development of a disease which

conforms closely to that type of smallpox in man which follows deliberate inoculation of the skin with variola virus. In short, we can produce in the monkey and in the orang-utan the homologue of human *variola inoculata.* We have also shown that if the epithelium of the cornea or of the mucous membrane of the nasal, buccal, or oral cavity be chosen as the *locus* of inoculation the same type of disease is produced. The experiments upon which this section are based were devised in an attempt to reproduce in the monkey and in the orang-utan a disease having the clinical features of *variola vera.* We have sought to attain this end by changing the *locus* of inoculation and by subjecting the animals to conditions in which man contracts the disease. The experiments which we have chosen to give in detail will be grouped according to the manner in which we have sought to bring about the infection.

(*a*) *Inoculation of the tracheal epithelium, through a tracheotomy wound, with the contents of the variola vesicle.*—These experiments were performed to determine if the course of the disease would be modified by the initial lesion being seated upon the columnar epithelium of the trachea. The generally accepted hypothesis of smallpox in man supposes a "protopustule" in the respiratory tract, and we proposed to deliberately produce such a lesion by direct inoculation with variola virus.

No. 144. Adult male, *M. cynomologus.* Monkey anæsthetized with chloroform and a median incision made in the neck over the upper part of the trachea. Trachea exposed by blunt dissection. The upper ring of the trachea cut, and through the opening so made the epithelium on the posterior wall slightly scarified and inoculated with variola virus No. 167 (vesicle contents). The skin incision closed with silk sutures. After 4 days the operation wound showed a marked inflammatory reaction.

On the ninth day of the experiment 6 red, papular, elevations, 2 to 3 millimeters in diameter, were seen on the scrotum. On the next day papules and small vesicles were found on the face, trunk, and extremities. On the eleventh day of the experiment the lesions on the scrotum presented flat topped, umbilicated vesicles with an opaque center and surrounded by a bright red areola. The evolution of these lesions was comparable with that seen in the exanthem of a case of discrete *variola vera* in man. A distinct pit remained after the healing of the lesions. One lesion was found in the palm of the hand. The body temperature rose to 40° C on the sixth day of the experiment and remained elevated until the thirteenth day.

No. 139. Adult male, *M. cynomologus.* Inoculated in the same manner as the previous animal. A profuse general exanthem appeared on the seventh day of the experiment. Animal died on the ninth day. At autopsy extensive cellulitis of the neck about the operation wound. Smears from this region show many streptococci. *Mucous membranes.*—In cheek pouches about a dozen opaque, elevated, sharply circumscribed lesions, 3 to 4 millimeters in diameter and 1 millimeter high. One similar lesion on under surface of tongue. Similar though smaller lesions are found scattered over the surface of the œsophagus, some of which are eroded. In the trachea at the site of inoculation is a red, slightly elevated area, due apparently to thickening of the epithelium. *Viscera.*—No macroscopic lesions found. *Skin.*—Vesicles and pustules, from 3 to 4 millimeters in diameter, are

found scattered over the face, trunk, and extremities. The exanthem consists of over 100 lesions.

Histological examination.—Operation wound: Subcutaneous tissue contains much fibrin and finely granular precipitate. There is considerable necrosis of the underlying muscle. Polymorphonuclear leucocytes in all stages of degeneration are present in large numbers. Many small blood vessels are thombosed, and in them and in the surrounding tissue streptococci are demonstrable. The epithelium at the edge of the wound is much degenerated, but no evidence of a variolous process can be made out. However, cytoryctes were found in the cells of the thickened corium. *Trachea:* The epithelium is wanting in places and in other areas it is somewhat thickened. In many places the basement membrane is broken. The epithelial cells show various degenerations. In many groups of cells cytoplasmic phases of *Cytoryctes variolæ* are demonstrable. The vessels of the submucous tissue are much injected, and in one is a mass of fibrin in which are phagocytic cells containing streptococci. The tissue is infiltrated with polynuclear leucocytes. The endothelial cells of a capillary immediately below an area in which the epithelium shows cytoryctes are prominent and contain cytoplasmic phases of the parasite. *Skin:* The lesions of the exanthem present the characters which before have been described in similar lesions occurring in the course of *variola inoculata* in the monkey. *Mucous membrane:* The lesions in the cheek pouches consist of areas of degenerated epithelium in which groups of cells are stained red, the nuclei having lost their affinity for basic dyes. The protoplasm is finely granular, but the cell outlines are retained. Other cells are present whose staining properties are unimpaired, and in these are found various cytoplasmic phases of *Cytoryctes variolæ*. In the upper layers of the degenerated epithelium, particularly toward the edge of the lesion, the cells are separated by fluid. In some places the appearance is similar to that seen near the edge of a 4-day primary lesion in the skin. Polynuclear leucocytes are present in the epithelium and in the tissue beneath, although in the latter situation the reaction is much less intense than it is beneath a primary lesion of the mucous membrane. *Œsophagus:*—Small areas are present in which the epithelial cells are swollen and their nuclei shrunken. Evidence can be made out of accumulation of fluid in and between the cells. Cytoplasmic forms of *Cytoryctes variolæ* are present in many of the cells. Comparatively few polynuclear leucocytes are present in the lesion, and there is practically no reaction in the tissue beneath. *Seminal vesicles:* Focal lesions are present in the septa of the tubules. The lesions consist of collections of degenerated cells and fibrin which lie in the connective tissue stalks of the septa. Associated with this there is more or less degeneration of the adjacent epithelial cells. A few polynuclear leucocytes are present. Many of the epithelial cells contain cytoplasmic phases of *Cytoryctes variolæ*. *Testicle:* Normal. *Bone marrow:* No focal lesions are demonstrable. The lung, liver, spleen, and kidney show no lesions.

Summary.—From observation of the trachea in animals killed at various times after the inoculation it was seen that a distinct process occurs at the site of inoculation. Our data on this point seems to us insufficient to form the basis of a description of the stages in the evolution of the specific lesion in this situation.

A general exanthem was observed in 3 of the 4 monkeys of this series. The single animal which did not show an eruption died on the eighth day of the experiment. The exanthem appeared on the seventh day in 1 animal, on the ninth day in the other 2. The exanthem was very extensive and in the 2 animals in which it appeared on the ninth day

of the disease it passed through an evolution similar to that of the eruption of *variola vera* in man.

The constitutional reaction was very marked, the animals showing anorexia and weakness during the active stage of the disease. This marked constitutional reaction may be, in part, explained by the intensity of the pyogenic process in the operation wound. The temperature reaction was similar to that seen after skin inoculation, showing a marked rise on the sixth or seventh day.

The histological examination demonstrates that the lesion at the site of inoculation and the lesion of the exanthem on the skin and the mucous membranes was variolus in type. In 1 animal focal lesions were demonstrated in the seminal vesicles and they were shown to be specific.

The method of inoculation used in this series of experiments was abandoned, as it was impossible to exclude a variolous infection of the operation wound. Obviously if this were to take place the experiments would be merely repetitions of the skin inoculation complicated by a coincident infection of the trachea.

(*b*) *Inoculation of the epithelium of the trachea, without tracheotomy, with the contents of the variola vesicle.*—These experiments were devised to overcome the difficulty experienced in the previous series resulting from infection of the tracheotomy wound. This series consists of experiments on 10 monkeys (*M. cynomologus*), of which 1 will be described in detail. Six animals were killed at various times after the inoculation to obtain material for histological study.

No. 339. Adult male, *M. cynomologus*. Monkey anesthetized with chloroform and a large glass tube, having a fire burnished end, introduced into the larynx through the mouth. By way of this tube instruments were introduced to scratch the wall of the trachea and to inoculate it with the virus. Variola virus No. 328 was employed.

On the seventh day of the disease the body temperature rose to 41° C., and a pink, paular elevation 1 millimeter in diameter appeared on the scrotum. On the next day small vesicles, surrounded by a bright-red areola, were present upon the face, scrotum, hands, and feet. On the following day, the ninth of the experiment, new eruptive lesions were found on the face, the scrotum, and the palms and soles. Animal chloroformed and autopsied at once. Skin lesions as described above. The mucous membrane of the trachea is congested throughout and presents several opaque spots on which are minute granular elevations. The trachea and large bronchi contain much slimy mucus. The left lung presents an area of consolidation, 5 millimeters in diameter, about the primary bronchus. On section, this area is reddish-brown in color and finely granular. Other organs appear normal.

Histological examination.—The microscopic study of the organs from these monkeys yielded data upon the variolous lesion in situations not previously described. The following descriptions are selected as types of the lesions found:

No. 345. *Trachea.*—Lesion of 3 days' duration. The basement membrane is intact. There is no reaction in the submucous tissue. The epithelial cells in a small area, which can be included in a single field of the oil immersion lens, contain various stages of the cytoplasmic forms of *Cytoryctes variolæ*. Aside from the vacuole about the parasite these cells appear normal.

No. 348. *Trachea*.—Lesion of 6 days' duration. The epithelium is thickened in places and frequently tongue-like projections, composed of epithelial cells, project into the lumen. There is wide-spread infection of the epithelial cells with cytoplasmic phases of *Cytoryctes variolæ*. In places groups of epithelial cells have lost their selective staining affinity and are colored an even red. Occasionally there is evidence of the collection of fluid within and between the degenerated cells. Polynuclear leucocytes are numerous and are found in the epithelium as well as in the submucous tissue. In places there is necrosis and accumulation of fibrin in the submucous tissue, such areas occurring beneath breaks in the basement membrane. The endothelial cells of the lymphatics and blood vessels of the submucosa are prominent and frequently contain cytoplasmic forms of *Cytoryctes variolæ*. *Lung*, 6 days after inoculation. The section shows a large bronchus and the surrounding lung substance. The epithelium of the bronchus is much thickened at 2 points in its circumference. In these areas the cells are more or less swollen and degenerated. The majority of the cells contain cytoplasmic forms of *Cytoryctes variolæ*. Polynuclear leucocytes are present in large numbers in the lumen of the bronchus, in the epithelium, and in the peribronchial tissue. The air cells for a considerable distance from this bronchus are filled with cells, fibrin, and granular precipitate. The relative amount of each of these constituents varies in different air cells, but in general the granular precipitate predominates in those remote from the bronchus. The cellular elements present are in the main polynuclear leucocytes and epithelial cells. The latter are found free in the air cells and also attached to the walls. In the latter situation they are frequently cuboidal in form and lie one beside another. Mitoses are frequently present in these cells. Cytoplasmic phases of *Cytoryctes variolæ* are present in large numbers in the epithelial cells of the air spaces. The capillaries in the septa between the affected air cells are injected with blood, and many polynuclear leucocytes are to be seen in them and migrating through their walls.

Two other monkeys, killed 7 and 9 days after inoculation, showed small areas of pneumonia in their lungs. In both cases the process was characterized by proliferation of the epithelial cells lining the alveoli. No cytoryctes were demonstrable in these lesions.

The exanthem which occurred in this series of monkeys was examined histologically and found to be similar in all respects to that which developed in the other variolated monkeys.

Summary.—Four animals were allowed to survive long enough to show an exanthem. Six were killed at various times after the inoculation in order to inspect the initial lesion and to obtain material for histological study. In each of the former animals an exanthem appeared; in 1 on the eighth day, in 2 on the ninth day, and in 1 on the tenth day. The exanthem was of moderate extent and showed an evolution intermediate between that in the monkeys inoculated through a tracheotomy wound and the average case of *variola inoculata* following variolation of the skin. The constitutional reaction was marked. The temperature reaction was definite, consisting of a distinct rise simultaneously with or immediately before the appearance of the exanthem.

The histological examination of tissues from these animals shows that a lesion similar to that which follows variolation of the mucous membrane of the nose, the lip, and the palate can be produced by inoculation of the epithelium of the trachea. In one animal a variolous bronchitis

and a variolous pneumonia was demonstrable. No focal lesions were found in the bone marrow or testicle.

A series of five Java monkeys (*M. nemistrinus*) was inoculated in the same manner and with the same virus. The results of these experiments were unsatisfactory. No exanthem or constitutional reaction was observed and we were unable to demonstrate a specific lesion at the site of inoculation.

(*c*) *Inoculation of the lung by inhalation of a spray of the contents of the variola vesicle.*—These experiments were performed to approximate the conditions under which natural infection with smallpox in man might be supposed to occur. Of course, the dose of virus is much larger than it possibly could be under natural conditions, but at least this method approaches more nearly epidemic conditions in that no deliberate trauma to an epithelial surface precedes the distribution or application of the contagium. Five animals were employed in this series, of which the following is selected to be described in detail:

No. 207. The monkey's mouth was held open and a fine spray of vesicle contents (virus No. 199) was thrown into the throat from an atomizer. The animal was observed to breath while the spray was acting.

No distinct temperature reaction was noted during the 17 days during which the animal was kept under observation. No lesion was observed to develop on the visible mucous membranes. On the seventh day of the disease a papule appeared on the inner aspect of the right arm, at the bend of the elbow. This lesion increased in size, became vesicular, and on the tenth day was distinctly umbilicated. The contents of this lesion was used to inoculate a fresh monkey upon the skin of the abdomen. A typical primary lesion developed and was followed by a profuse general exanthem.

Summary.—One of the animals of this series was killed 5 days after inoculation. Inspection of the mucous membrane showed no evidence of a specific lesion. Sections from various parts of the lungs and the trachea were studied microscopically, but no lesions were found. The 4 animals remaining were under observation for 16 days. In one of them a single eruptive lesion appeared on the seventh day of the experiment. This lesion was shown to be specific by inoculation of its contents upon the skin of a fresh monkey.

No constitutional reaction or rise of temperature was observed in this series of monkeys.

(*d*) *Inoculation of the lung by inhalation of dry variola virus.*—These experiments were devised to determine if *variola vera* could be produced in the monkey by employing the contagious material in a dry condition. Two series of experiments were performed, in one dried pustule contents was used and in the other a powder prepared from desiccated crusts or disks of a case of *variola vera*.

A preliminary experiment was performed to determine how far a powder blown into the larynx would enter the lung. A large monkey was anesthetized with chloroform and a glass tube containing a mixture

of lycopodium and methylene blue powder was introduced into the larynx. During an inspiration the powder was forcibly blown into the lungs. The animal was killed at once and, on dissection, the smallest bronchi which could be made out with the naked eye were found to be stained distinctly blue. Histological examination of the lung showed lycopodium spores in the bronchi and the alveoli.

No. 160. A monkey was put under chloroform anesthesia and the same procedure was followed as above described, except that dry pustule contents was substituted for the methylene blue. The animal was kept under observation for sixteen days. The monkey had a cough from the fifth to the eighth day after the inoculation. On the eighth day of the experiment the body temperature rose to 40°.5 C., and an abundant general exanthem appeared. The lesions of the eruption passed through an evolution which closely simulated that seen in the lesions of a discrete *variola vera* in man. On the sixteenth day of the experiment the animal was vaccinated on the abdomen with virus No. 1. No reaction followed.

Two other monkeys were inoculated in the lung in the same manner and with the same virus. One of these developed symptoms like that above described. The third animal showed a distinct rise of temperature on the third day of the experiment and was immune to subsequent vaccination but did not develop an exanthem. The same method of inoculation was followed in another series of 5 monkeys, but powdered variola disk was substituted for the mixture of pustule contents and lycopodium.

Summary.—Two of the animals in the series inoculated with dried pustule contents showed an exanthem. The exanthem appeared on the eighth day of the disease and was profuse. It one of these animals the evolution of the eruption was similar to that seen in *variola vera* in man. A cough was noted in the 2 monkeys which developed an exanthem. The constitutional reaction was not marked, but in each animal a distinct rise in body temperature was observed. The fever began on the eighth day in two and on the sixth day in one.

The monkeys inoculated by inhalation of pulverized variola disks showed no exanthem, no constitutional reaction, and an indefinite temperature reaction. One animal was found to be refractory to a subsequent skin inoculation with variola virus.

(*e*) *Attempts to inoculate the monkey by exposure to smallpox fomites.*—In the series of inoculations previously described we employed material the infectiousness of which was demonstrable. In all these experiments, although they approximate somewhat the conditions in which man contracts smallpox, the amount of contagious material employed was excessive. In the series to be described we attempted to place the animals under conditions which experience has shown would result in an attack of smallpox if man was subjected to the test, although we were unable to demonstrate the presence of a contagium.

No. 243. Adult male, *M. cynomologus*. This monkey, together with 4 others, was kept in a cage in which a blanket was placed which had been wrapped around a smallpox patient. The blanket had been in contact with the patient for 6 hours

and was carried from the ward to the animal room in a light-proof, sterile bag. The blanket was left in the cage with the monkeys over night. Observations were recorded upon the monkeys daily for a period of 16 days, during which time no eruption and no temperature reaction appeared. The animal was variolated on the skin on the seventeenth day of the experiment with virus No. 260. A typical primary lesion developed at the site of inoculation.

One of the remaining animals of this series died on the fifteenth day of the experiment, before the immunity had been tested by skin inoculation. At autopsy there was no evidence of variola in this animal. The 3 other monkeys were shown to be susceptible to variolation by skin inoculation.

Summary.—Five monkeys showed no clinical symptoms of variola after exposure to a smallpox-infected blanket. Four of these animals were subsequently shown to be susceptible to variola by skin inoculation. The fifth animal died before its immunity was tested, but no anatomical evidence was found that it had contracted variola.

(*f*) *Attempts to inoculate the monkey by exposure to a smallpox patient.*—This experiment was undertaken as a further test of the reaction of the monkey to conditions which would bring about an infection with smallpox in man.

No. 240. Adult male, *M. cynomologus.* This monkey and 4 others were placed in a cage which was kept in a room for 16 hours with a smallpox patient in the vesicular stage of the disease. The animals were kept under observation for 16 days. No eruption and no temperature reaction was observed. On the seventeenth day of the experiment the animal was inoculated on the abdomen with variola virus No. 260. A typical primary lesion developed at the site of inoculation.

Of the 4 remaining monkeys of this series 2 reacted positively to subsequent variolation on the skin while 2 gave no reaction.

Summary.—Five monkeys exposed to a smallpox patient did not develop symptoms of variola. Three of these animals were subsequently shown to be susceptible to variolation by skin inoculation.

(*g*) *Attempt to inoculate by exposure of the orang-utan to smallpox fomites.*—This experiment was planned to determine if an animal higher in the scale than the monkey would contract smallpox under conditions in which man becomes infected.

No. 197. A young female orang-utan was given a blanket which had just been taken from a case of smallpox in the vesicular stage of the disease. The animal at once wrapped herself in the blanket and used it until the following day, when a clean blanket was substituted for the infected one. During the following eighteen days the animal was kept under constant observation. No exanthem appeared and the variations in the body temperature which occurred were explained by intercurrent infection. The animal was subsequently variolated on the skin and yielded a positive reaction.

Summary.—An orang-utan exposed to smallpox fomites did not develop symptoms of variola. The animal was shown subsequently to be susceptible to *variola inoculata* by skin inoculation.

DISCUSSION.

The experiments detailed above were devised to produce *variola vera* in the monkey or in the orang-utan. An analysis of the results of these experiments shows that of 29 monkeys 10 developed a distinct group of symptoms. Seven of the remaining animals showed no symptoms and 12 were affected in an indefinite way. All the positive results were obtained where some product of the cutaneous lesion of human smallpox was introduced into the animal. All of the negative results were in experiments where smallpox fomites or the air of a smallpox ward was depended upon to carry the contagium. Fifteen experiments in which vesicle contents or pustule contents were either inoculated upon the tracheal mucous membrane or blown into the lung yielded 10 positive results. The disease produced in these animals was characterized by the development of an exanthem, some degree of constitutional reaction, and fever. The evolution of the exanthem was usually similar to that seen in *variola inoculata*, but in 3 animals it resembled the eruption in a mild *variola vera*. The exanthem appeared between the seventh and tenth days of the experiment. The constitutional reaction and the fever appeared on the sixth, seventh, or eighth day of the experiment.

The disease which occurred in these animals agrees with that type which follows variolation of the skin of the monkey, and we have no difficulty in recognizing it as *variola inoculata*.

The negative results which followed exposure of the monkey and the orang-utan to smallpox fomites and smallpox patients show that these animals do not develop a recognizable form of variola when placed under conditions which we believe would produce smallpox in man. We are unable to exclude the possibility of the occurrence of *variola vera sine exanthem* or *variola inoculata sine exanthem* in these animals. The 2 monkeys which were refractory to variolation after exposure to a smallpox patient might owe their immunity to such unrecognizable forms of variola, but we are inclined to regard this phenomenon as very likely due to an individual peculiarity of the animals (natural immunity).

The histological study of the tissues from these series of monkeys brings out certain points of interest. The epithelium of the trachea is shown to be capable of harboring the parasite of the disease, and we see that a lesion can develop in this location which has features in common with the ones produced by variolation of other mucous membranes.

The occurrence of a variolous lesion in the bronchus and, associated with it, a pneumonia in which the parasite is present shows that the organism is capable of multiplying in the deeper parts of the respiratory tract. This fact bears upon the pathogenesis of *variola vera* in man. It is quite conceivable that such a lesion might run its course unnoticed and serve as a focus for the multiplication of the organism during the incubation period of the disease. We have shown that *Cytoryctes variolæ* is capable of infecting the endothelial cells of capillaries and

lymphatics. It is easy to understand that the organisms in a focus of variolous pneumonia might invade the adjacent blood vessels, infect endothelial cells, and so be carried throughout the vascular system. If such an infected endothelial cell were to lodge in a capillary adjacent to an epithelium hospitable to the organism, an eruptive lesion would result. The focal lesions in the seminal vesicles of one of the monkeys which was inoculated in the trachea is doubtless due to such a process, although in this case the infected endothelial cells probably came from vessels beneath the tracheal inoculation. In *variola inoculata* the tissue at the site of the inoculation is flooded with organisms at the time of inoculation, and it seems probable to us that the exanthem developing in these animals results from endothelial cells infected at this time. In *variola vera* the exanthem appears four or five days later than in *variola inoculata*. We believe this interval to be associated with the development of a focus of variolus pneumonia, during which the organisms multiply and finally invade the vessels.

CONCLUSIONS.

(1) Inoculation of the mucous membrane of the trachea of the monkey (*M. cynomologus*) with variola virus produces a *variola inoculata* in that animal.

(2) Inhalation of variola virus by the monkey (*M. cynomologus*) produces a *variola inoculata* in that animal.

(3) Exposure of the monkey (*M. cynomologus*) and the orang-utan (*Simia satyrus*) to smallpox fomites and to a smallpox patient does not produce *variola vera* or any other recognizable form of variola in these animals.

(4) Inoculation of the moucous membrane of the trachea of the monkey (*M. cynomologus*) with variola virus is followed by the development of a variolous lesion on the mucous membrane which is similar to that produced on other mucous membranes by similar inoculations. A variolous lesion may develop in the bronchi and be associated with a pneumonia in which *Cytoryctes variolæ* are present. The development of the specific lesion in the trachea may be followed by a general cutaneous exanthem and also by focal lesions of a variolous nature in the seminal vesicles.

(5) *Cytoryctes variolæ* can invade the epithelial cells of the trachea, the bronchi, the alveoli of the lung, and the seminal vesicles.

(6) *Cytoryctes variolæ* can invade the endothelial cells of lymphatics and blood vessels. This property of the organism plays an important part in the production of the exanthem in variola.

PART III.

STUDIES UPON THE IMMUNITY REACTIONS OF THE MONKEY AFTER INOCULATION WITH VACCINE OR WITH VARIOLA VIRUS.

I. ON THE IMMUNITY REACTIONS OF THE MONKEY (MACACUS CYNOMOLOGUS) AFTER INOCULATION OF THE SKIN WITH VACCINE OR WITH VARIOLA VIRUS.

In this section we propose to bring together certain observations upon the immunity following the development of vaccinal or variolous lesions upon the skin of the monkey. The data bear upon the general problems of immunity to vaccinia and variola, and we will show certain differences in the immunity reactions of the monkey to the two sorts of virus which throw light upon the general question of the interrelationships of the two diseases. The experiments which form the basis of this section were in part those detailed in other papers of this series and in part the ones performed with special reference to the problems here treated.

The technique used for inoculation was that described in previous papers of this series. The diagnosis of the results of the second inoculations was based upon the descriptions already given of the specific lesions of vaccinia and *variola inoculata* in the monkey. We have been guided wholly by the appearances to the naked eye. In our experience we have rarely been in doubt as to the specific character of a vaccinal or variolous skin lesion in the monkey. In the few instances where we could not feel certain of the diagnosis, the experiment has been ruled out.

EXPERIMENTS.

1. *Vaccination of the skin after successful vaccination of the skin.*—Thirteen monkeys were selected for this experiment. Each animal had shown a typical vaccinal lesion of the skin of the abdomen from inoculation either with virus No. 1, 148, 236, 246, or 251. Twenty-two days after the occurrence of this lesion the animals were vaccinated on the skin of the abodmen with virus No. 148. All these attempted revaccinations resulted negatively. At the site of the second inoculation there was only the usual, slight reaction which follows a scratch. The scratches healed as if no virus had been used.

2. *Variolation of the skin after successful vaccination of the skin.*—Six monkeys which had shown typical lesions on the skin of the abdomen as

the result of inoculation either with vaccine virus No. 1, 251, 236, 246, or 148 were selected for this experiment. Each animal was variolated on the skin of the abdomen with virus No. 52 or 200. No reaction followed the second inoculation. An interval of from 38 to 58 days elapsed between the two inoculations.

3. *Vaccination of the skin after variolation of the skin.*—The results obtained in this series are best shown by presenting a number of the experiments in detail.

No. 114. Adult male, *M. cynomologus.* Variolated on the skin of the abdomen with virus No. 167 (vesicle contents). Animal developed a typical *variola inoculata* including a general exanthem. On the thirty-seventh day after the variolous inoculation the monkey was vaccinated on the skin of the abdomen with virus No. 148. At the site of inoculation there developed a lesion which had all of the characteristics of a vaccine process only differing from primary vaccinations in the extent of the process and in the indefiniteness of the vesiculation. The lesions passed through a definite evolution and healed spontaneously. We had no hesitation in diagnosing the lesions as specific but typical reactions.

The experiment was repeated on 2 other monkeys of the same species with identical results, which need not be given in detail.

No. 153. Adult male, *M. cynomologus.* The monkey was variolated in a number of places on the skin of the abdomen with virus No. 167 (vesicle contents). A typical, primary lesion developed, which, however, was not so extensive as in the other animals inoculated at the same time with the same virus. No exanthem was noted.

On the tenth day of the experiment the animal was vaccinated on the skin of the abdomen with virus No. 148. An atypical reaction resulted, similar to those seen in the monkeys described above.

On the twenty-fifth day the monkey was vaccinated on the skin of the abdomen with virus No. 1. An atypical lesion was again produced.

No. 141. Adult male, *M. cynomologus.* Variolated on skin of abdomen with virus No. 167 (vesicle contents). *Variola inoculata,* with exanthem, developed. Twenty-eight days after the variolation the animal was vaccinated on the abdomen with virus No. 1. No reaction followed the vaccination.

Two other monkeys were shown to be immune to vaccination of the skin with virus No. 1, 19 days after a variolation on the abdomen with virus No. 167 (pustule contents dried with lycopodium), which had been followed by a typical primary lesion but no general exanthem.

4. *Variolation of the skin after variola inoculata.*—This experiment was only undertaken in one instance and demonstrated the monkey to be immune, after a *variola inoculata,* to a second skin inoculation with variola virus.

SUMMARY.

(1) Vaccination of the skin in 13 monkeys protected against subsequent vaccination of the skin.

(2) A vaccination of the skin in 6 monkeys protected against subsequent variolation of the skin.

(3) A variolation of the skin in 3 monkeys protected against subsequent vaccination of the skin. In the case of 3 monkeys the following vaccination yielded a positive, though an abortive, reaction. Another

monkey showed an abortive reaction with 2 successive vaccinations which were subsequent to a variolation.

(4) Variolation of the skin in 1 monkey protected against a subsequent variolation of the skin.

(5) The time which elapsed between the first and second inoculation in these monkeys varied between 10 and 58 days.

(6) Three of the monkeys which were shown to be susceptible to vaccination after successful variolation were tested 37 days after the first inoculation. The 3 animals of the same series which were refractory to the vaccination after *variola inoculata* were tested 28 days after the primary inoculation. The animal which did not seem to acquire an immunity to inoculation (No. 153) was tested on the tenth and twenty-fifth days—that is, previous to the date in which complete immunity was shown to exist in 3 monkeys and to the date in which 3 were shown not to be immune to a second inoculation.

DISCUSSION.

The results of our inoculations conform to the general law that vaccination and variolation confer an immunity to subsequent infection with vaccine and variola virus upon the affected animal. The results of similar inoculations in man, which were performed in the early days of vaccination, seem to have yielded more constant results than we have obtained in monkeys. The immunity conferred by a vaccine lesion of the skin of the monkeys is complete against later inoculation with vaccine and variola virus.

The conclusions are not so definite in primary variolation. In a certain proportion of the animals a complete immunity to vaccination on the skin has been produced by a previous *variola inoculata*, but an equal number show only a diminished susceptibility to the vaccine virus. These observations agree with those made by Roger and Weil on Macacus monkeys, in which substantially the same phenomenon was noted.

In seeking for an explanation of this partial immunity conferred by *variola inoculata* against vaccination of the skin we might refer it simply to a dying out of the immunity, for we find the completely immune animals were reinoculated on the twenty-eighth day of the experiment, while the animals showing partial immunity were tested on the thirty-seventh day; but this is contradicted by the single animal which was shown to react by a specific process to 3 successive inoculations, with an interval of 10 and 15 days, with variola and vaccine virus (experiment No. 153).

The explanation that there is a qualitative difference in the reaction of this species of animal to the 2 viruses is not borne out by experimental inoculation. The inoculation of variola virus affords partial protection in a certain percentage of cases, and absolute protection in others, to subsequent vaccination.

A third possibility lies in a hypothetical quantitative difference in the immune substance called forth by the 2 sorts of virus.

It seems evident that the immunity which the animal presents to the skin inoculation must depend upon certain properties of the individual conferred upon it by the disease which follows the first inoculation. The weight of evidence is in favor of the immunity being due to a bactericidal or germicidal property resident in the blood serum (Sternberg and Reed, Béclère, Chambon, and Menard). If such be the case the animals in which complete immunity to vaccinia follows variolation, and in which complete immunity to variolation follows vaccination, indicate that the immune property of the serum of the inoculated animal, whether vaccination or variolation be practiced, is identical. We then would expect to find simply a quantitative difference in this germicidal property of the sera of the animals, depending upon the character of the virus used for inoculation. The reason that *variola inoculata* always protects against an inoculation with variola virus, if confirmed by more experimental evidence than we present, would be that the variola virus is less potent upon the monkey than vaccinia virus, in that it fails to develop in monkeys protected by previous variolation, whereas the latter develops and produces a lesion.

That the immunity resulting from inoculation of the monkey with variola virus is less efficient than that resulting from vaccination is apparent from the fact that vaccination protects against both subsequent variolation and vaccination, while variolation protects against subsequent variolation and only partially against subsequent vaccination.

At the present time technical difficulties prevent the putting of the quantitative aspects of the above hypotheses to the test of experiment.

CONCLUSIONS.

(1) A vaccine lesion on the skin of the monkey (*M. cynomologus*) confers upon the animal an immunity to subsequent inoculation of the skin with vaccine or with variola virus.

(2) A variolous lesion on the skin of the monkey (*M. cynomologus*) protects the animal against subsequent inoculation of the skin with variola virus, but does not in all cases protect against later inoculation with vaccine virus.

(3) The failure of *variola inoculata* in the monkey to protect against subsequent skin inoculation with vaccine virus depends upon the fact that this species of animal produces a smaller amount of the germicidal substance necessary to inhibit a second inoculation after variolation than it does after vaccination.

2. ON THE INFLUENCE OF THE LOCUS OF INOCULATION UPON THE DEVELOPMENT OF THE IMMUNITY IN VARIOLA AND VACCINIA IN THE MONKEY (MACACUS CYNOMOLOGUS).

Introduction.—In the preceding section we have studied the immunity reactions of the monkey to inoculation of the skin with variola and with vaccine virus and have brought out certain differences in the immunity

produced by the two viruses. In this section we will detail experiments which bear upon the general problem of the immunity reactions of the monkey to vaccine and to variola virus from a somewhat different point of view.

In testing the immunity of rabbits after skin and after corneal inoculations with vaccine virus, Dr. R. L. Thompson[7] obtained results which tended to show a difference in the degree of immunity depending on the *locus* chosen for the primary vaccination. As we were more favorably situated as regards facilities and animals for experimentation, we decided to continue this line of work on monkeys. We have extended the scope of the experiments so as to include the study of the relative immunity produced by vaccination and variolation of the skin, cornea, and mucous membranes.

EXPERIMENTS.

(*a*) *Vaccination of the cornea after vaccination of the skin.*—This experiment was performed upon 5 monkeys (*M. cynomolgus*). Each animal had shown a typical vaccinal lesion on the skin as a result of an inoculation with virus No. 1, 148, 236, or 251, and each was tested 22 days after the first inoculation by vaccination of the skin with virus No. 148. The cornea was vaccinated with virus No. 148 on the twenty-ninth day after the initial skin vaccination. The animals were killed after 48 hours, and paraffin sections of the corneas were examined microscopically.

Four of the monkeys showed no specific lesion on the cornea. One showed a typical vaccinal keratitis with proliferation of the epithelium and the presence of numerous cytoryctes.

(*b*) *Variolation of the cornea after variola inoculata from skin inoculation.*—This series consisted of 5 monkeys which had shown a typical primary lesion on the abdomen following inoculation with variola virus No. 167 (disk). Three of these animals had developed a general exanthem.

On the twenty-fourth day of the experiment each monkey was inoculated on the cornea with variola virus No. 167 (vesicle contents). The animals were killed after 72 hours and the corneas studied microscopically. Each animal presented a typical variolous keratitis at the site of inoculation, and cytoryctes were present in large numbers.

(*c*) *Variolation of the skin after variolous keratitis.*—This experiment was performed on a single animal. The cornea was inoculated with variola virus No. 200. A typical lesion developed. Eighteen days after the corneal variolation variola virus No. 252 was inoculated on the skin of the abdomen without producing a lesion. The skin inoculation was repeated on the forty-first day with variola virus No. 307, and again no reaction followed.

(*d*) *Vaccination of the skin after variolation of the mucous membrane of the palate.*—The 5 monkeys employed for this experiment had developed a typical variolous lesion on the soft palate, following variolation with virus No. 167 (vesicle contents). Each animal was vaccinated on the skin with virus No. 148

[7] These experiments were carried on in the pathological laboratory of the Boston City Hospital under the direction of Dr. W. T. Councilman. Owing to the impossibility, at the time, of carrying out the research on extensive enough lines to yield definite conclusions, the results were not published.

on the twenty-first day of the experiment. In every animal a typical vaccine lesion developed at the site of inoculation.

(*e*) *Variolation of the skin after variolation of the mucous membrane of the palate.*—Three monkeys were selected that had shown a typical variolous lesion on the soft palate after inoculation with virus No. 307. Eighteen days after the initial inoculation the animals were variolated on the abdomen with virus No. 307. Two of the monkeys showed no reaction to the skin inoculation, while 1 yielded a typical primary lesion, but no exanthem developed.

(*f*) *Vaccination of the skin after variolation of the mucous membrane of the lip and nose.*—One monkey was inoculated in this way. Typical variolous lesions developed on the inner side of the lip and on the nasal septum after inoculation with variola virus No. 167 (vesicle contents). The monkey was vaccinated on the abdomen with virus No. 148 and No. 1 on the seventeenth and forty first days, respectively. No lesion developed after either vaccination.

(*g*) *Vaccination on the skin of the temple after vaccination of the abdomen.*—Two monkeys that had shown a typical vaccine lesion on the abdomen were later shown to be refractory to vaccination on the temple.

SUMMARY.

(*a*) A vaccine lesion of the skin protects against subsequent inoculation of the cornea with vaccine virus, but the protection is not complete.

(*b*) A variola lesion of the skin does not protect against subsequent inoculation of the cornea with variola virus.

(*c*) In one monkey a variola lesion on the cornea protected against subsequent inoculation of the skin with variola virus.

(*d*) A variola lesion on the mucous membrane of the palate does not protect against subsequent inoculation of the skin with vaccine virus.

(*e*) A variola lesion on the mucous membrane of the palate does not completely protect against subsequent inoculation of the skin with variola virus.

(*f*) A variola lesion on the mucous membrane of the lip and nose protected, in one instance, against subsequent inoculation of the skin with vaccine virus.

(*g*) A vaccine lesion on the skin of the abdomen protected against subsequent inoculation of the skin of the temple with vaccine virus.

DISCUSSION.

The summary of our experiments in this section demonstrates that the immunity produced by a variolation on the mucous membrane is lower than that produced by a variolation on the skin.

We have already shown that the immunity produced by a variolation on the skin is lower than that following a vaccination on the skin, and we find that this is emphasized by the results of inoculations of the cornea after vaccination and variolation of the skin. The fact that even vaccination of the skin does not completely protect against subsequent corneal inoculation with vaccine virus illustrates our point with regard to the quantitative relation of the two immunities.

In interpreting the results of these inoculations the following factors must be considered, viz:

(*a*) The *locus* of the initial inoculation.

(*b*) The virus used in the initial inoculation.

(*c*) The *locus* of the second inoculation.

(*d*) The virus used in the second inoculation.

Bearing these factors in mind we see that *loci* chosen for initial inoculations bear the following relation to the resulting immunity.

The immunity conferred by skin *locus* is greater than that by the cornea, and the latter is greater than that by the mucous membrane. The immunity conferred by vaccine is greater than that conferred by variola, as we have already indicated in the preceding section.

The influence of the *locus* of the second inoculation can only be estimated in regard to the cornea and skin when we find that the immunity conditioned by the initial inoculation is less efficacious when the cornea is chosen than when the skin is the site of the second inoculation.

The influence of the sort of virus used in the second inoculation upon the test seems to indicate that the vaccine virus is more potent than the variola virus in that it may produce a lesion in an animal in which there is complete protection to inoculation with variola.

We find, in short, that the skin is relatively a more efficacious *locus* than the cornea, and the latter is more efficacious than the mucous membrane in producing immunity. The immunizing power of vaccine virus is higher than that of variola virus.

If we interpret the above experiments in the light of the hypothesis elaborated in the previous section and keep in mind the physical conditions at the various *loci* of inoculation, we feel that the phenomena observed are quite consistent.

A variolation of the cornea after skin variolation succeeds because the total amount of immune substance present in the individual is relatively small, owing to the character of the virus used in the initial inoculation, and because the physical conditions on the cornea do not favor a free mixing of the immune-bearing plasma with the inoculated virus.

In the case of a monkey vaccinated on the skin a vaccination of the cornea only rarely succeeds, because the relatively large amount of immune substance present, even under the adverse physical conditions in the cornea, usually is sufficient to produce a germicidal effect upon the inoculated virus. We feel that the fact of an occasional animal yielding a positive reaction to such a second inoculation only emphasizes our view that the phenomenon is a quantitative one.

In the case of the mucous membrane as a *locus* of initial inoculation the physical conditions are doubtless a large factor in causing the low immunizing power. Study of the lesion on the mucous membrane shows that almost from the first an open wound is present at the site of inoculation. This condition would favor the discharge of toxine and products

of degeneration of the organism, and would be unfavorable for the production of an immune substance which resulted from the reaction of the host cells.

The high potentiality of the skin as a *locus* of the initial inoculation for immunity production is in sharp contrast with that of the mucous membrane, and the physical conditions are in keeping with the interpretation given above. In a skin lesion the greater part of the products of the lesion must be absorbed and go to produce the general immunity.

It seems probable from the histological study of the specific lesion in the nose that this *locus* would stand nearer to the skin in its potentiality for immunity production than the lip or palate. We feel that our data are insufficient for generalization on this point.

CONCLUSIONS.

(1) The degree of protection conferred by a vaccinal or variolous lesion on the monkey (*M. cynomologus*) is conditioned by the *locus* chosen for inoculation as well as by the virus which is employed.

(2) The varying degree of immunity production which follows the development of vaccinal or variolous lesions at different *loci* of inoculation is dependent upon the physical conditions there present.

(3) The outcome of an inoculation of an animal which has had a variolous or vaccinious lesion depends upon the *locus* and upon the virus employed in the second inoculation as well as upon the *locus* and upon the virus employed in the first one.

3. ON THE TIME OF DEVELOPMENT OF THE IMMUNITY AFTER INOCULATION OF THE SKIN OF THE MONKEY WITH VACCINE AND WITH VARIOLA VIRUS.

Introduction.—The following experiments were planned to show the interval which elapses between the inoculation of the monkey's skin with vaccine or variola virus and the development of an immunity inhibiting further inoculations. The results of these experiments bear upon the general problem of the diseases, and particularly upon that of the causation of the exanthem in *variola inoculata*. The experiments are arranged in three series, as follows:

(*a*) *Daily inoculation of the skin with vaccine virus.* Five monkeys (*M. cynomologus*) were selected and each received upon the skin of the abdomen a single vaccination daily for 9 days. The development of each lesion was observed and objective descriptions recorded from day to day. Vaccine virus No. 148 was employed.

No. 108. The lesions from vaccinations performed on the second, third, fifth, sixth, and seventh days of the experiment showed a typical development. Those from inoculations on the first and fourth days were abortive in character, vesiculation not being complete. The inoculations done on the eighth and ninth days were entirely negative.

No. 109. The first, second, third, fourth, and sixth vaccinations were positive, while no reaction followed those on the fifth, seventh, eighth, and ninth days of the experiment.

No. 110. The first to the fifth vaccinations, inclusive, resulted positively, while those on the 4 succeeding days were not followed by a reaction.

No. 111. The first to the fifth vaccinations, inclusive, yielded a typical vaccine process, while the remaining 4 were without result.

No. 112. The first, second, and third vaccinations were negative, the fourth was abortive, and the fifth, sixth, seventh, eighth and ninth were negative.

(*b*) *Daily inoculation of the skin with variola virus.*—Eight monkeys (*M. cynomologus*) were used in this series of experiments. Each animal daily received a single inoculation with variola virus. Variola virus No. 167 (vesicle contents), No. 199, or No. 200 was employed, and after the final inoculation the sample was tested by inoculation upon the skin of a fresh monkey and shown still to be potent.

No. 136. The inoculations on the first, second, third, fourth, and fifth days of the experiment yielded typical lesions. The fifth, sixth, and seventh inoculations were not followed by a lesion. A general exanthem developed on the ninth day of the experiment.

No. 137. The first to the fourth inoculations, inclusive, were positive, while the remainder were negative. An exanthem appeared on the ninth day.

No. 138. The first 4 inoculations were positive, which the last 3 were negative. An exanthem developed on the eighth and ninth days.

No. 213. The first, second, third, and fourth inoculations were positive, while the fifth, sixth, and seventh were negative. On the seventh, eighth, and ninth days a general exanthem was observed.

No. 214. The inoculations on the second and third days yielded positive, those on the first and fourth abortive, and those on the fifth, sixth, and seventh negative reactions. No general exanthem developed.

No. 215. The first four inoculations were positive, the fifth was questionable, the sixth and seventh were negative. No exanthem developed.

No. 116. The first, second and third inoculations were positive, the fourth was questionable, the fifth, sixth, and seventh were negative. A general exanthem appeared on the eighth and ninth days.

No. 117. The first four inoculations were positive, the fifth was questionable, the sixth and seventh were negative. An exanthem appeared on the ninth day.

(*c*) *Simultaneous inoculation with vaccine and variola virus.*—Five monkeys were selected and inoculated on the left groin with vaccine virus No. 1, and on the right side of the chest with variola virus No. 200.

Four animals reacted typically to both inoculations and 2 of these developed an exanthem on the ninth day of the experiment. The fifth monkey reacted typically to the vaccine virus, but did not show a process at the site of the inoculation with variola virus or develop an exanthem.

SUMMARY.

(1) In 2 monkeys daily inoculations with vaccine virus ceased to produce a positive lesion 5 days after the first inoculation.

(2) Three monkeys similarly vaccinated failed to react 6 days after the first successful inoculation.

(3) In 4 monkeys inoculated daily with variola virus positive reactions were not obtained 4 days after the first inoculation.

(4) In 2 monkeys inoculated daily with variola virus, an abortive lesion followed the inoculation done 4 days after the first one, and in 2 animals a similar lesion developed from an inoculation performed 3 days after the primary inoculation.

(5) Six of the 8 monkeys subjected to daily inoculations with variola virus developed an exanthem. The eruption was first present on the ninth day in 2, on the eighth day in 3, and on the seventh day of the experiment in 1.

(6) The interval between the last successful daily inoculation (counting abortive lesions as positive) and the appearance of the exanthem was 5 days in 2 monkeys, 4 days in 3, and 3 days in 1.

(7) Four monkeys reacted to both vaccine and variola virus, simultaneously inoculated, by the appearance of typical lesions which developed apparently without influencing one another. Two of these animals developed a general exanthem on the ninth day of the experiment.

DISCUSSION.

A comparison of the results of daily inoculation of the skin with vaccine virus with those following similar inoculation with variola virus shows that there is a distinct difference in the time of onset of the immunity.

In the experiments where vaccine was used the refractoriness to skin inoculation, if judged by the day on which the first unsuccessful inoculation was performed, appeared, on an average, during the seventh day of the experiment—that is to say, the seventh daily inoculation, which was performed 6 days after the first successful one, fails to show a specific reaction.

In the series where variola virus was employed this refractoriness to reinoculation appeared, on an average, during the fifth day of the experiment.

Without committing ourselves to the exact date of onset of the immunity, we may yet assert from this that it is of earlier development in *variola inoculata* than in vaccinia. The determination of the exact day of development of an immunity to subsequent skin inoculation can not accurately be determined by this procedure. In studying the evolution of a vaccine or a variola lesion on the skin, we see that an interval of from 72 to 96 hours intervenes between the inoculation and the appearance of a process which can be diagnosed by the naked eye.

That this incubation period is apparent rather than real, as shown by a microscopic study of sections from the inoculation sites, does not help us in this connection. By the methods adopted in these experiments we are in doubt for 3 days as to the outcome of our inoculation. At any time during this period the development of the lesion may be checked by the onset of the immunity. We then see that the fact that the inoculations on the first 4 days of an experiment result in a diagnosable lesion, while those on the fifth day and on succeeding days do not develop to a diagnosable condition, does not fix the date of onset of immunity at the fifth day. Obviously an inoculation done on the fifth day might not appear in the records of the experiment as a positive reaction if the immunity developed even 2 days later, as the lesion would be inhibited before it was recognizable.

Therefore we must conclude that the onset of the immunity is not before the date of the last successful inoculation, and may be as much as 3 days later. Applying this conclusion to our experiments, where daily inoculation was practiced, we see that the development of immunity to reinoculation of the skin with vaccine virus may manifest itself at any time between the sixth and the eleventh days, and to reinoculation with variola virus between the fifth and the eighth days. These figures are arrived at by selecting the days of the earliest unsuccessful inoculation for the earliest date and the day of the latest unsuccessful inoculation plus three for the latest date. These limits, while wide, are as narrow as we believe to be warranted by the method of experimentation adopted.

The appearance of a general exanthem in the animals inoculated daily with variola virus, from 3 to 5 days after the last successful insertion of virus on the skin, seems at first sight difficult to explain. If we allow for a 3-day interval between the invasion of the skin and the appearance of the eruptive lesion, we see that this brings the date of invasion, and hence the period of intravascular transit of the organisms to within the limits set for the onset of the immunity. The number of organisms which go to produce a regular, eruptive lesion are undoubtedly infinitely fewer than those introduced in an inoculation of the skin with virus. As the growth of the lesion depends upon the multiplication of the organisms, it is probable that the interval between the invasion of the skin and the appearance of the exanthem lesion is longer than that between inoculation of the skin with virus and the diagnosable stage of the primary lesion. We must conceive of the organisms which are to produce the exanthem as passing from the primary lesion to the skin before the date of onset of the immunity. By comparing the dates we can readily understand how the organisms might make this intravascular journey before the immunity developed. Another explanation lies in the possibility that phagocytes act as carriers and as protectors of the organism from the immune plasma. The development of an exanthem is therefore quite consistent with our conclusions with regard to the time of onset of

the immunity. The brief evolution and abortive development of the lesion of the exanthem is what might be expected in an animal which had already developed such a germicidal power in its plasma that its presence in the inoculation wound, and in the subsequent exudate stream of inflammatory origin, inactivated the virus introduced at the site of a skin inoculation.

In the lesion developing spontaneously on the skin, the immune plasma doubtless does not have as free access to the organisms as is the case where the virus is mixed in a scratch with fresh drawn blood serum.

The phenomenon of an exanthem in *variola inoculata* and its absence in vaccinia is not explained by these experiments. Had it proved that the general immunity was of notably later development in *variola inoculata* the exanthem producing quality of variola virus would readily have been explained. However, the reverse seems to be the case, and we have to seek further for the explanation of this fundamental difference between the two viruses.

Simultaneous variolation and vaccination of the monkey shows that the synchronous development of a vaccine lesion has no effect upon the appearance of the exanthem of *variola inoculata*. The fact that vaccination on or about the date of exposure to smallpox inhibits the production of clinical types of *variola vera*, characterized by an exanthem, emphasizes the difference between the diseases *variola vera* of man and *variola inoculata* of monkeys.

In a previous section we have shown that the immunity potential of the mucous membrane is low. In *variola vera* it seems exceedingly probable that the atrium of infection and the site of the primary lesion is on a mucous membrane. If such be the case we would expect that little if any immunity would develop as a result of the evolution of this lesion, and the organisms which seek the skin to produce the exanthem would develop in a practically nonimmunized animal. This agrees with the course of the exanthem in a typical *variola vera* in man. In other cases of smallpox the exanthem shows an evolution very like that in *variola inoculata* (*variola abortiva*), or is absent (*variola sine exanthem*), and in these we conceive the organisms which go to form the exanthem as acting against a more or less fully developed immunity. This condition might be dependent upon an early development of the general immunity arising from the primary pock, and be conditioned by its *locus*. In any case, a vaccination on the skin at the time of exposure produces an immunity which develops before the exanthem, probably killing the organisms in transit from the protopustule to the skin, and so inhibiting the eruption.

This suggests an explanation of the failure of all attempts to abort the exanthem in *variola vera* by the injection of what certainly were highly germicidal sera (Béclère and others).

It is evident that at the time when the case is fully declared to be smallpox and is put under treatment, the organisms are inaccessible

to the serum. To be effective the serum would have to be given before the disease had advanced to a diagnosable state. Such sera might be useful in cases where a patient well advanced in smallpox is discovered in an unvaccinated family. The unprotected ones in contact with such a case will probably be in the incubation stage of smallpox, and might be protected by injections of serum from a *variola vera* with exanthem.

CONCLUSIONS.

(1) The immunity which accompanies the development of a vaccine lesion on the skin of a monkey becomes manifest between the sixth and eleventh days.

(2) After a variola lesion of the skin the immunity appears between the fifth and eighth days.

(3) The organisms which produce this exanthem in *variola inoculata* in the monkey pass from the point of inoculation to the skin before the onset of the general immunity.

(4) The development of an exanthem in *variola inoculata* in the monkey is not dependent upon a late development of the immunity reaction of the animal.

(5) The use of variolicidal sera is indicated only in cases where it can be administered during the incubation stage of the disease.

PART IV.

ON THE OCCURRENCE OF CYTORYCTES VARIOLÆ GUARNIERI IN EXPERIMENTAL VARIOLA AND VACCINIA IN THE MONKEY AND IN THE ORANG-UTAN.

In a previous publication from the Department of Pathology of the Harvard Medical School, the specific inclusions found in the cells in vaccinia and variola, which have been the subject of investigation by Guarnieri and others,[8] were described at length, and the conclusion was reached that they were intracellular organisms. In addition to the bodies in the protoplasm, which are common to the lesions of vaccinia and of variola, a series was found within the nuclei of the epithelial cells in the specific lesions of variola. These intranuclear bodies were first described by Councilman, Magrath, and Brinckerhoff, and were regarded by them as phases of the organism peculiar to smallpox.

In the present paper the name "Cytoryctes" will be applied to the specific nuclear as well as to the specific cytoplasmic forms. Since the morphology of these various forms has fully been described by a number of observers,[9] this phase of the problem will not be treated in this paper, attention being directed to the occurrence and distribution of these inclusions in various lesions produced experimentally in monkeys and in the orang-utan.

Literature.—Since the preliminary report on variola by Councilman, Magrath, and Brinckerhoff,[10] Bose has published a series of articles relating to smallpox and vaccinia. He describes in vaccinia and in variola cytoplasmic forms which possess definite structure and which pass through a developmental cycle resulting in multiplication. In variola he describes an additional cycle within the nucleus, and the forms pictured are similar to those discovered by Councilman. He believes these structures to be the causal agent in the disease.

Ewing (7) considers the cytoplasmic forms of cytoryctes as products of degeneration of the cytoreticulum, in support of which he cites the intimate relation of the bodies to the reticulum of the cell. The cycle presented by the various forms he believes to be one of degeneration

[8] For literature to 1904 see *Journal of Medical Research*, **11**, 116.

[9] Councilman, Magrath, and Brinckerhoff (3), Calkins (1), Howard and Perkins (9), Bose (4) (5).

[10] Councilman, Magrath, and Brinckerhoff (2), May, 1903.

rather than development, and is unable to distinguish certain features, such as nuclear structure, spore formation, and multiplication, which characterize the well-known protozoan parasites.

Ewing finds the nuclear forms specific to smallpox, but does not consider that sufficient proof has been adduced to identify them as organisms. He finds them identical in staining reaction with nucleoli and linin globules and claims to demonstrate transitions between the latter and the larger vacuolated forms. The presence of these structures in cells advanced in degeneration he regards as against the parasitic theory.

Howard and Perkins (9) confirm the life cycle of cytoryctes as presented by Calkins and present a hitherto undescribed secondary cytoplasmic stage. They believe that it is this form which first invades the nucleus and gives rise to the nuclear phases previously described.

Ewing (8) in a more recent publication presents results obtained by the application of the *Klatsch* method of making histological preparations. He claims that by this method there is less artefact, especially as regards shrinkage, than in tissue fixed and sectioned by the usual methods. After applying this technique to the study of vaccine lesions of the cornea and skin he concludes that the vaccine body is a portion of the cytoreticulum, the alteration in its staining reaction being due to a diffusion of nuclear proteids into the cytoplasm. He suggests the possibility of the presence of specific organisms within these "degenerations." He admits the vaccine bodies to be specific to variola and vaccinia.

Siegel (11) confirms previous descriptions of the cytoplasmic forms, but suggests that the nuclear forms described by Councilman are post-mortem artefacts. He bases this inference on the statement of Tyzzer that he was unable to find these intranuclear forms in perfectly fresh tissue, and utterly disregards the experimental work on monkeys in which the presence of the nuclear bodies was shown in perfectly fresh tissue.

Davidson (6) studied the tissues of a case of variola and found the cytoplasmic but no nuclear forms.

Shrumpf (10) does not consider either the nuclear or cytoplasmic inclusions to be organisms. His conclusions do not seem to be based upon very extensive studies.

Technique.—The details of inoculation in the series of experiments from which our material for this study was obtained are given in full in other sections of this paper and need not be repeated. However, attention is called to the fact that the method of making multiple inoculations upon the skin furnished lesions of sufficient number to excise one at each 24-hour interval from the time of inoculation until repair had begun. By this method it is possible to obtain all stages of the process in each animal. In the study of the disease produced in other ways than by inoculation upon the skin, animals of a given series were killed at regular

intervals, usually at 24-hour periods, from the time of the inoculation and the lesions and tissues fixed for histological study. All tissue was at once put in Zenker's fluid for fixation and afterwards embedded in paraffin by the alcohol-chloroform method. Sections having a thickness of about 5 microns were cut, except in a few instances when sections of one-half this thickness were prepared. The Mallory eosin-methylene-blue method of staining gives very satisfactory results for the study of cytoryctes and was generally used. A large number of the staining methods now in common use were tried, but none was found to be specific for cytoryctes.

The lesions upon which this histological study is based were obtained chiefly from Philippine monkeys (*M. cynomologus*) inoculated with vaccine or with variola virus. These were supplemented by variola lesions of the Java monkey (*M. nemestrinus*), variola lesions of the orang-utan (*Simia satyrus*), and primary vaccinations from the human subject. The material furnished by the various series of experiments is as follows:

1. *Vaccine lesions of the skin.*—Fifteen Philippine monkeys each with multiple vaccinations: A lesion excised at each 24-hour interval from 1 to 10 days after inoculation. Eleven other monkeys killed at various periods after vaccination. Two vaccine lesions of the human subject obtained 5 and 7 days, respectively, after vaccination.

2. *Vaccination of the cornea.*— Nine monkeys furnishing lesions of 17, 24, and 48 hours, and 3, 4, 5, 7, and 8 days' duration.

3. *Vaccinations of mucous membrane.*—Nine monkeys vaccinated upon the inner surface of the lip, on the nasal septum, and on the soft palate. Killed at 24-hour intervals from 1 to 9 days.

4. *Primary variola lesions of the skin.*—Ten monkeys each receiving multiple inoculation: A lesion excised each day as in the skin vaccination series. Eleven other Philippine monkeys killed at various intervals after inoculation. Three Java monkeys: Lesions excised at 24-hour intervals from 3 to 9 days after variolation. An orang-utan furnished lesions 6, 7, and 8 days after variolation.

5. *Variolations of the cornea.*—Nineteen monkeys.

6. *Primary variola lesions of the mucous membrane.*—Nine monkeys variolated on the inner surface of the lip, on the nasal septum, and on the soft palate: Killed at 24-hour intervals from 1 to 9 days. Five monkeys variolated on the nasal septum alone: Killed 2, 3, 4, 5, and 6 days after the inoculation.

7. *Primary variola lesions of the trachea.*—Eight monkeys. In one of these there was also a specific process in the lung, possibly an extension from the trachea.

8. *Variola exanthem.*—Lesions appearing at various locations upon the skin after an interval of 6 to 10 days after inoculation with variola. The eruption of one case in which it was profuse upon the mucous membrane of the oral cavity and œsophagus as well as upon the skin. In this case there were also lesions of a specific nature in the seminal vesicle which were considered to be a part of the eruption.

This material furnishes data on the occurrence of cytoryctes in lesions produced by the inoculation of three species of apes with variola, and in vaccine lesions of the Philippine monkey and of man.

Cytoplasmic forms are found in every lesion in which there is a characteristic process following the inoculation of vaccine. In the vaccination lesions of the skin, cytoryctes are usually demonstrable 48 hours after inoculation and in a few instances 24 hours after inoculation. They persist in the lesions up to 8 days after the vaccination and may occasionally be found in small numbers for a longer period. They are found at the sides of the vesicles where there is a gradual transition from normal epithelium to that in which degeneration is advanced. The small, deeply stained, and sharply defined forms are found at the periphery where the epithelium is nearly normal. The expanded indefinite forms which Calkins has described as ameboid forms, and residual masses from which the granules have disappeared are found in older portions of the lesions. These indefinite forms are present in lesions of 72 hours and in some cases in those of 48 hours duration. In the early lesions they are found in relatively small *foci*, but as the process advances they are distributed through a greater mass of the epithelium. They are often distinguishable in cells advanced in degeneration, but are not so in the fluid of the vesicle. In several lesions cytoryctes were found in the endothelium and in other cells about a small superficial blood vessel, just beneath the vesicle.

The process produced by the vaccination of the cornea is accompanied in every instance by the cytoplasmic forms of cytoryctes. With the extensive destruction of epithelium in these inoculations, a considerable surface is denuded so that cytoryctes are present only in small numbers at the edge of the degenerating epithelium.

In the vaccine lesions of the lip, nose, and soft palate cytoryctes are constantly present. Their distribution is similar in these lesions to that of the skin lesions, the small, sharply defined forms occurring at the periphery and the larger expanded forms in the older portion of the process.

In this large number of vaccine lesions produced by the inoculation of monkeys with 4 different strains of vaccine virus, the nuclear forms of cytoryctes as found in variola do not occur. However, in vaccine lesions it is not uncommon to find nuclei distended with eosin-stained material in the form of reticulum, hyaline granules or globules. In some cases inclusions of this sort are abundant. The eosin-stained masses may have a regular, definite contour, or they may be faint and indefinite in outline. The reticular masses usually have an irregular or frayed periphery. Greatly distended nuclei may contain a large number of rounded globules of uniform size, which in some instances show a tendency to vacuolation, or in others may possess deeply staining centers. These inclusions often contain masses of deeply stained material, evidently the chromatin of the epithelial cells. However, vaccine lesions of the skin, the cornea, and the mucous membrane of the lip, nose, and soft

palate, taken at all stages of the process from its beginning up to the time of repair, fail to show the presence of the specific nuclear bodies found in variola. Two primary vaccine lesions from a native child, obtained at 5 and 7 days, respectively, after vaccination, present no nuclear forms such as are found in variola lesions.

THE OCCURRENCE OF CYTORYCTES VARIOLÆ IN PRIMARY VARIOLA LESIONS.

Primary lesions produced by the inoculation of Philippine monkeys (*M. cynomologus*) with variola virus are constantly associated with the cytoplasmic forms. The distribution of these forms in variola skin lesions differs in one respect from their distribution in vaccine lesions of the skin. In the process which is present beneath the epithelium in the primary variolation, and which involves the dermis and subcutaneous tissue, cytoryctes are present in large numbers. They are found with endothelial cells *in situ* within the vessels or lying in the adjacent tissue. The endothelium of blood vessels as well as lymphatics is thus affected. Three Java monkeys (*M. nemenstrinus*) afforded skin variola lesions of a peculiar type. The process affects but a small area of the epithelium, while the dermis and subcutaneous tissue show an extensive lesion. Cytoplasmic forms are present in the endothelial cells found in this region, also in sections in which they are not demonstrable in the epidermis.

The time occurrence of the cytoplasmic forms is practically identical with that in vaccine lesions of the skin. They are present in lesions of 48 hours, and in some instances in those of 24 hours' duration. They usually disappear from the lesion 9 days after inoculation, but occasionally they are found subsequent to this in small foci. Their distribution in the epithelial portion of the lesion is similar to that in vaccine lesions. The nuclear forms of cytoryctes are found in the majority of lesions resulting from variolation of the skin of both the Philippine and the Java monkey. In no case are these forms numerous. In many the study of a single section through the lesion reveals one of several of these forms. A prolonged search through a great many sections is necessary in other cases to find a single typical nuclear form. They are usually found in cells forming the floor of the vesicle or in those of the hair follicles where the degeneration of cells is advanced. Associated with the typical nuclear forms are other inclusions which, however, are not specific to variola. These appear as masses of stringy reticulum, hyaline bodies, granules, and globules within the nuclei, similar to the forms already described in vaccinia. They may include portions of the nuclear chromatin and may all be duplicated in vaccine lesions, but are not nearly so abundant there as in the variola lesion. The reaction of these structures to the eosin-methylene blue stain is apparently identical with that of the specific nuclear forms.

In the skin variolations, hyaline masses within the nuclei may show a tendency to vacuolation or they may have definite rings embedded in

their substance. Intranuclear forms of cytorcytes were found in variolations of the skin from 3 to 8 days after inoculation. As they were so uncertain in their occurrence one could not well judge at what time they were most numerous. In all cases they were preceded by the cytoplasmic forms and were never found without these.

In primary variola lesions of the orang-utan taken 6, 7, and 8 days after inoculation, nuclear forms are very abundant. A variety of both the specific forms and the nonspecific reticular and hyaline bodies may be found in a single section. In this respect the skin variolations in the orang-utan differ greatly from those of the two species of monkey employed, which show relatively few nuclear forms. Cytoplasmic forms are present in these variola lesions of the orang-utan. Their distribution is similar to that in the preceding species.

In the lesions following variolation of the monkey's cornea the cytoplasmic forms are constantly present but never the nuclear forms. The cytoplasmic forms appear rather more numerous than in vaccinations of the cornea, probably on account of the retention of the epithelium. They are found in lesions of 24 hours after inoculation and are present in those of 11 days' duration, beyond which time we have no material. This nonoccurrence of intranuclear forms is probably due to physical conditions.

In the primary inoculations of the lip, the nose, and the palate the occurrence and distribution of both cytoplasmic and nuclear forms are essentially the same as those found in skin variolations. In one instance the nuclei of the nose lesion were filled with the nonspecific eosin-staining inclusions, but there were but few typical intranuclear bodies.

In lesions of the trachea produced by inoculation with variola virus cytoplasmic forms are present. In one case a specific variolous pneumonia accompanied the inoculation of the trachea and cytoryctes were present within the epithelial cells lining the alveoli. In this case the process appears to have extended from the bronchus into the lung substance.

THE OCCURRENCE OF CYTORYCTES VARIOLÆ IN EXANTHEM LESIONS.

A general eruption followed the inoculation of various sites upon the body with variola virus. Eruptions were produced by the inoculation of the skin, the cornea, the mucous membrane of the lip, nose, and palate, the trachea, and by intravenous injection of variola virus. The lesions of the eruption appear after periods of from 6 to 10 days subsequent to inoculation.

Cytoplasmic forms are found constantly in these eruption lesions, excepting in those in which repair is advanced. Except in the exanthem resulting from intravenous injection, the specific nuclear forms are rare. However, they are found in the exanthem following skin inoculation in several cases, and it is probable that by a study of a greater number of lesions and a longer series of sections they would be shown to occur in a large number of cases. The exanthem following intravenous

injection furnishes a greater number of nuclear forms than any other experimental lesion, with the exception of the primary lesion of *variola inoculata* in the orang-utan; in this respect it approaches the character of the *variola vera* eruption in man.

Besides the lesions appearing as an eruption on the surface of the body following inoculation with variola virus, similar ones may appear on the mucous membranes, or in more remote organs. Following the inoculation of the tracheal epithelium of a monkey through an incision in the neck, there was not only a profuse eruption upon the skin, but likewise upon the mucous membrane of the mouth, cheek pouches, and œsophagus, and also in the seminal vesicles. In all these lesions the cytoplasmic forms of cytoryctes are present.

THE OCCURRENCE OF CYTORYCTES VARIOLÆ IN REINOCULATIONS.

In many animals which had been inoculated upon the skin with variola virus, a subsequent inoculation of the cornea with variola or vaccine virus resulted in a typical reaction. The corneal lesions in these cases contain cytoryctes which can not be distinguished from those of the lesions of first inoculations.

Lesions produced by the inoculation of dried smallpox or vaccine virus appeared later and ran a milder course than was the case with fresh virus. In these lesions cytoryctes were present in small numbers, this apparently being due to the limited area affected. They were morphologically identical with those occurring in other lesions.

SUMMARY AND CONCLUSIONS.

(1) The cytoplasmic forms of *Cytoryctes variolæ* are found constantly in all specific lesions resulting from inoculation with variola or with vaccine virus. They appear in the primary lesions of both *variola inoculata* and vaccinia soon after the inoculation. They persist in the primary skin lesions for about 8 days after inoculation, at which time immunity is established and repair is beginning. In *variola inoculata* the exanthem as well as the primary lesions contain cytoplasmic forms.

(2) Intracellular forms are found within the epithelial nuclei in lesions resulting from the inoculation of the monkey with variola virus and do not occur in vaccine lesions. These structures are specific for variola. Other nonspecific, nuclear inclusions occur in vaccinia, in variola, and in other nonrelated processes.

(3) The nuclear forms of cytoryctes, which are found only in small numbers in the primary skin lesion of *variola inoculata* in the monkeys (*M. cynomologus* and *M. nemenstrinus*), are present in far greater numbers in the corresponding lesion of the orang-utan.

(4) Nuclear forms were only occasionally found in lesions of the general eruption following the inoculation of the skin of the monkey with variola virus, but were very numerous in the eruption which followed the intravenous injection of variola virus into the tail vein,

with subsequent amputation of the tail proximal to the point of inoculation. This eruption resembles in this, as well as in other respects, the eruption of *variola vera* in man.

(5) The cytoplasmic forms of cytoryctes are constantly associated with variola and vaccinia in whatever portion of the body the lesions may develop. Thus they are found included in a variety of cells, the squamous epithelium of the skin, the cornea, the mucous membrane of the nose, oral cavity, and œsophagus, the cells of the sebaceous and Meibomian glands, the epithelium of the conjunctiva, the columnar epithelium of the nose, trachea, and seminal vesicle, the epithelium lining the alveoli of the lung, endothelial cells, and connective-tissue cells.

(6) The occurrence of cytoryctes in the cells of the corium, and especially within the endothelium of vessels, suggests a possible method of dissemination of the organism in the production of the exanthem. However, endothelial cells containing cytoryctes were also found in a few instances in vaccine lesions, a form of the disease never accompanied by a general eruption.

(7) The occurrence and distribution of the specific inclusions is best explained by the hypothesis that they are parasites, and that as such they are the cause of the disease.

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PART V.

ON THE REACTION OF VARIOLA VIRUS TO CERTAIN EXTERNAL CONDITIONS.

Introduction.—In the experiments described in the other parts of this report we have been engaged with the reactions of the inoculated animal to the contagium of smallpox and of vaccinia. In this section we propose to emphasize the changes which the contagium itself undergoes under certain experimental conditions.

The contagium of variola is demonstrable in the specific skin lesions of human smallpox and in the lesions produced in various animals by inoculation with such material. The proof of the presence of the contagium depends upon the results of the inoculation of a suitable animal with the suspected material, as no cultural or micro-chemical technique has been devised which permits of the certain identification of the organism, save in sections of the specific lesion. This being the case, we have only limited criteria for estimating the quality of a given sample of virus. By inoculation of the monkey we can determine whether or not the virus will produce the typical variolus lesion, and if it does do so, we can classify the lesions produced by different strains of virus according to the course of development of the primary lesion, the occurrence and extent of the exanthem, and the degree of constitutional reaction. From these data we may draw certain inferences as to the quality of the samples of virus tested. We give below the results of such tests of various samples of virus which had been treated in such wise as to modify their properties.

(*a*) *The effect of keeping upon variola virus.*—No. 71. A sample of virus collected from a case of smallpox, at autopsy, in the pustular stage, produced a positive reaction when inoculated on the abdomen of a monkey (*M. cynomologus*). The virus was placed in an ice box where it was exposed to a varying temperature, which at times reached many degrees above freezing. Fifty-seven days later the virus was found to have a putrid odor. Inoculations on the abdomen of a monkey and on the cornea of a rabbit yielded no reaction of any sort.

Another sample of virus became putrid after a few days keeping, yet yielded typical takes when inoculated on the monkey's skin. On many occasions vesicle contents, sealed in capillary tubes and kept on ice, was found potent after three weeks.

No. 44. Variola disks collected from cases of the epidemic in Boston during February, 1902, were tested sixteen months later. The material had been subjected to the high temperature of the steamer's hold during the journey across the Pacific. Inoculations on the monkey's skin and on the rabbit's cornea were negative.

No 48. Vesicle contents collected from a case in the Philadelphia epidemic during January, 1902, sealed in capillary tubes and exposed to the same conditions as the Boston disks, was found inactive when inoculated on the rabbit's cornea 4 months later.

(*b*) *The effect of drying upon variola virus.*—This series of experiments was undertaken to test the resistance of variola virus to drying at room temperature. For comparison, parallel experiments were made with vaccine virus.

The ends of a number of glass rods were coated with a thin film of vesicle contents from a case of smallpox. Similar preparations were made from the five-day vaccine lesions of a calf. The rods were at once put into sterile test tubes, closed with cotton plugs, and kept at room temperature.

After two weeks the rods coated with vaccine virus produced typical lesions on the monkey's skin when rubbed upon a fresh scratch. The rods coated with variola virus were inactive. After three weeks the variola rods were again tested and found inactive. A final test of the vaccine rods three and a half weeks after preparation showed them to have also become inactive.

(*c*) *The effect of passage through the Berkefeld filter upon variola virus.*—A sample of variola virus (No. 199, vesicle contents) was shown to be active when inoculated upon the skin of monkeys. A portion of the virus was filtered through a small Berkefeld filter, "N". The filtrate was inactive when inoculated on the skin of 2 monkeys and on the cornea of 1 rabbit. The monkeys used in this test were later shown to be susceptible to skin inoculation with variola virus. The unfiltered portion of virus No. 199 was shown to be active for many days after the test of the filtered portion.

(*d*) *The effect of glycerin upon variola virus.*—In a previous publication[11] we stated that we had found variola virus to be rendered inactive by mixture with glycerin. The following tests were made to control the results of our preliminary experiments along this line:

No. 252. A large amount of variola virus (vesicle contents) was collected from a case of smallpox at autopsy. The material was put on ice and after 24 hours a portion of it was mixed with 60 per cent glycerin in the proportion of 1 part virus to 3 parts glycerin. The mixture was thoroughly shaken and kept on ice. The glycerinated virus was tested from time to time by inoculation on the monkey's skin.

No. 255. Monkey inoculated on the abdomen with the glycerinated virus after 3 days on ice. A typical primary lesion developed, but its evolution was somewhat delayed. An exanthem consisting of 2 typical lesions appeared on the tenth day.

No. 273. Monkey inoculated on the abdomen with the glycerinated virus after 11 days on ice. A typical primary lesion developed and was followed by an exanthem consisting of 3 small vesicles on the tenth day.

No. 318. Monkey inoculated as before, with glycerinated virus which had been on ice for 26 days. A typical primary lesion developed which was delayed in its evolution and was not followed by an exanthem.

No. 363. Monkey inoculated as before with the glycerinated virus which had been kept on ice for 50 days. The primary lesion was typical but delayed in its evolution. No exanthem developed.

[11] *Jour. Med. Research*, 11, 296.

(*e*) *The effect upon a strain of variola virus of repeated passages through the monkey.*—No. 200 virus. A series of 5 monkeys was inoculated with this strain of virus, each animal save the first being inoculated with curettings of the primary lesion of the previous monkey of the series. Typical primary lesions developed on each animal. The exanthem was profuse after the first and second serial inoculation, but was sparse in the third and fourth animal. The strain was transferred to 2 additional monkeys from the primary lesions of the second monkey of the series, and in each of these animals an atypical primary lesion developed, but was not followed by an exanthem. After the third serial transfer the strain was inoculated on another monkey, yielding an atypical primary lesion followed by a sparse eruption. A transfer from the primary lesion of this animal resulted in an atypical primary lesion and no exanthem.

A series of 4 monkeys was inoculated by transfer of virus from the exanthem, beginning with the second animal of the first series described. Each animal showed a typical primary lesion save the last, in which the process was somewhat delayed. A profuse exanthem developed in each animal.

The strain was inoculated at each transfer of the above series to another monkey and in each case yielded an atypical primary lesion. The first transfer was followed by a moderately extensive exanthem.

The second animal of the series of inoculations with the virus from the exanthem was used as a source of virus for testing the immunity of certain monkeys. The contents of the primary lesion was employed. Three animals inoculated with this virus developed typical primary lesions, which were followed by a sparse general exanthem.

No. 199 virus. This strain of virus was carried through one orang-utan and two monkeys. The first monkey showed a typical primary lesion and a profuse exanthem. The second monkey did not react to the inoculation.

The time of the occurrence and the evolution of the exanthem in all the monkeys of these experiments was similar to that seen in animals inoculated with fresh human variola virus.

SUMMARY.

(1) A variola virus (vesicle contents and disk) lost its power to produce a specific lesion upon the skin of the monkey after prolonged exposure to high temperature, to putrefaction, and to desiccation.

(2) A sample of variola virus lost its power to produce a specific lesion on the monkey's skin and on the rabbit's cornea by passage through the "N" Berkefeld filter.

(3) A sample of variola virus retained its power to produce a primary lesion, but lost its exanthem-producing properties after exposure to 60 per cent glycerin for 50 days.

(4) A strain of variola virus showed less and less power to cause a typical primary lesion on the monkey and a waning exanthem producing potentiality after repeated passages through monkeys.

DISCUSSION.

The reaction of vaccine virus to drying and to the action of glycerin has been carefully determined by those who have charge of the preparation of vaccine for protective inoculations against smallpox. It is a matter of common knowledge that vaccine retains its potency for a

comparatively long time in the dry state. Our experiments with variola virus are not very extensive and we hesitate to draw broad conclusions from them, but so far as they go they show that variola virus is less resistant to drying than vaccine. This whole question of the comparative resistance of the two sorts of virus to physical conditions is unsettled and presents an attractive field for study.

In our earlier experiments we found that variola virus did not retain its potency in the presence of 60 per cent glycerin. As the results of our later experiments upon this point show variola virus will resist the action of glycerin for a considerable period, we are inclined to believe that our first experiments were done with a glycerin which was not absolutely neutral, but that the inactivation of the virus was due to an acid in the glycerin and not to the action of the pure reagent. The loss of the power to develop an exanthem after prolonged contact with glycerin is of interest and should be made the basis of further work. It is important to determine whether this loss is associated with any change in the development of the two cycles of the parasite.

The question of the passage of the variola and vaccine contagium through the filter has been the subject of a certain number of experiments. We have only one observation to record upon this, which shows that the organism is held back by the filter. It is to be noted that this inactivation of the virus by filtration was tested by skin inoculations on the monkey and did not exclude the presence of diffusible contagium in the filtrate.

The influence of serial transfers upon the variola virus is shown in our series. This demonstrates that the virus tends to die out when transferred from monkey to monkey. This is in contrast with the stability of a strain of vaccine on an animal.

The question of the relative virulence of different strains of virus requires for its intelligent discussion the criteria for judging of the virulence of a given virus. In the case of variola virus the reactions of the animal inoculated consist in the development of a local lesion, the production of an exanthem, the tumefaction of the lymph nodes, and in some constitutional disturbance. The two former present many degrees of variation and seem most suitable as a basis for judgment as to the virulence of the virus inoculated. We can distinguish between various reactions at the site of inoculation and classify them as typical, atypical, abortive, etc. We can observe the occurrence, the extent, and the evolution of the exanthem. If we had only the virus to consider, we should classify our different strains or samples of virus according to the degree of local reaction at the site of inoculation and the nature of the exanthem. However, such a direct interpretation is impossible, as we must take into consideration the degree of natural immunity of the animal chosen for inoculation to a virus. In order to estimate this element it is necessary to compare the results of the inoculation of

several animals with the same sample of virus. If the majority of the animals yielded atypical or abortive lesions, we should be warranted in saying that the virus was avirulent. If, on the other hand, a majority of the animals gave typical lesions, we should classify the virus as virulent.

Applying this test to all our experiments, we find that certain strains of variola virus we have used may be classified as virulent and others as avirulent. We may also say that the transfer of a strain of variola virus from one monkey to another tends to reduce its virulence. It also seems to be the case that drying, either in the course of the disease, as in the formation of the crust, or artificially, reduces the virulence. In the same way, exposure to 60 per cent glycerin lowers the virulence.

This brings up the question of the nature of the difference between variola and vaccine virus. The two strains of virus may be said to bear a relation of virulence and avirulence to one another, so far as the results of our experiments go. However, there is an important difference between the two contagiums which we have not been able to bring out experimentally but which is a matter of common knowledge. This difference may be put as follows: Vaccine produces a local lesion at the site of inoculation, but no exanthem, and is not air-borne; variola produces a local lesion at the site of inoculation, an exanthem, and is air-borne.

The occurrence of an exanthem and of an air-borne contagium still needs explanation. In the extenuation of a strain of variola virus we see that the exanthem-producing potentiality is lost before the virus becomes entirely inactive. Variola virus which has lost its power to produce an exanthem has practically no points of difference from a vaccine virus, so far as its reactions on the monkey are concerned. Whether or not it has in fact become a vaccine virus could only be determined by inoculations and by exposure experiments with human beings. Such procedures are of course impossible and the solution of the problem must await the findings of an experimental animal in which *variola vera* can be produced under the same conditions which produce the disease in man.

CONCLUSIONS.

(1) Variola virus is less resistant to desiccation than vaccine virus.

(2) Variola virus does not pass through the "N" Berkefeld filter.

(3) Variola virus is attenuated by long exposure to 60 per cent glycerin. The virus so treated loses its power to produce an exanthem when inoculated on the skin of the monkey (*M. cynomologus*).

(4) Variola virus tends to die out when passed repeatedly through the monkey. The exanthem-producing power is lost before the virus has become incapable of producing a primary lesion.

ILLUSTRATIONS.

PLATE I.

FIG. 1. *Variola inoculata* in the orang-utan (*Simia satyrus*). Lesion at the site of inoculation. Duration 6 days. Wide spread vesicle formation extending outward from the inoculation wound.

2. *Variola inoculata in the orang-utan* (*Simia satyrus*). *Lesion at the site* of inoculation. Duration 7 days. Vesicle contains more fluid than before. Multilocular character of lesion well shown. False vesicle formation to the right of the inoculation wound.

PLATE II.

FIG. 1. *Variola inoculata* in the Philippine monkey (*Macacus cynomologus*). Lesion at the site of inoculation. Duration 6 days. Dark mass in center is the crust. On either side there is vesicle formation and at the periphery of the lesion thickening of the epidermis. Marked œdema and cellular infiltration of the corium beneath the lesion.

2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem in the skin of the palm of the hand. The lesion is well advanced in the process of healing.

PLATE III.

FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem. Vesicular stage. Well-marked cellular infiltration about the vessels of the corium.

2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem. Pustular stage.

PLATE IV.

FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem in cheek pouch. Animal was inoculated in the trachea.

2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Focal lesion in the seminal vesicle, of the nature of an exanthem. From same animal as in fig. 1.

PLATE V.

FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion at the site of inoculation in the nose. The process in a stratified epithelium.

2. Same as fig. 1, but inoculation is on a columnar epithelium. Note the cellular infiltration of the submucous tissue.

PLATE VI.

FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion at the site of inoculation on the trachea. Well-marked, tongue-like projections of the epithelium due to swelling and proliferation of the epithelial cells. This stage proceeds one in which the surface becomes denuded of epithelium. Duration 6 days.

2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Variolous lesions in the bronchus and in the lung following inoculation of the trachea. Variolous bronchitis and variolous pneumonia. Duration 6 days.

PLATE VII.

FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Protoplasmic phase of *Cytoryctes variolæ* in the epithelial cells of the alveoli of the lung in a variolous pneumonia.

2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Protoplasmic forms of *Cytoryctes variolæ* in the epithelial cells of the seminal vesicle.

PLATE VIII.

FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). *Cytoryctes variolæ*, protoplasmic forms in the epithelium of the trachea.

2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Protoplasmic form of *Cytoryctes variolæ* in an endothelial cell in a capillary beneath a lesion of the palate of 3 days' duration. (× 2000.)

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PLATE I.

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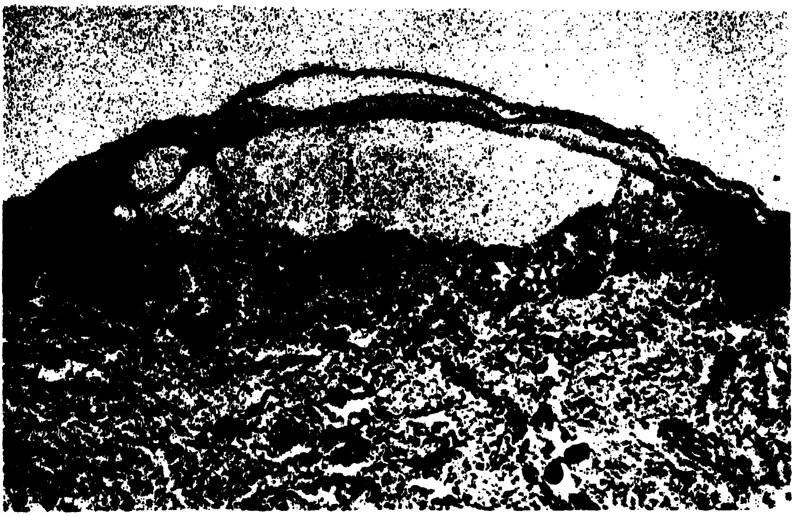
PLATE II.



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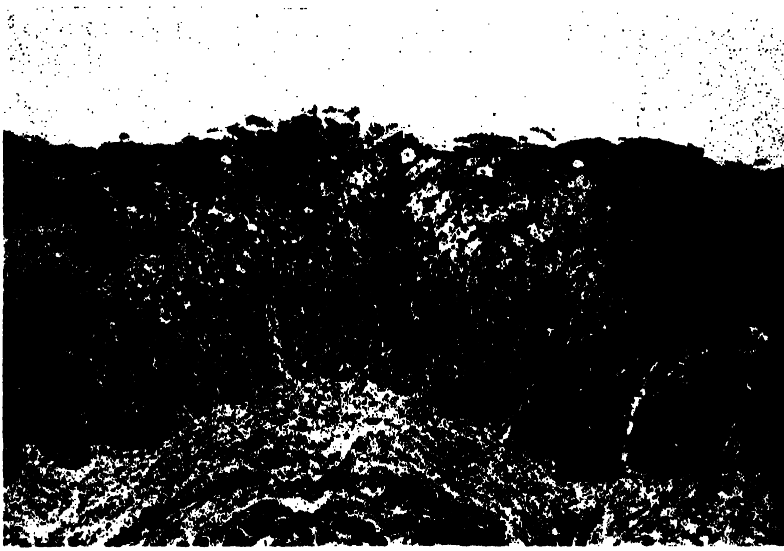

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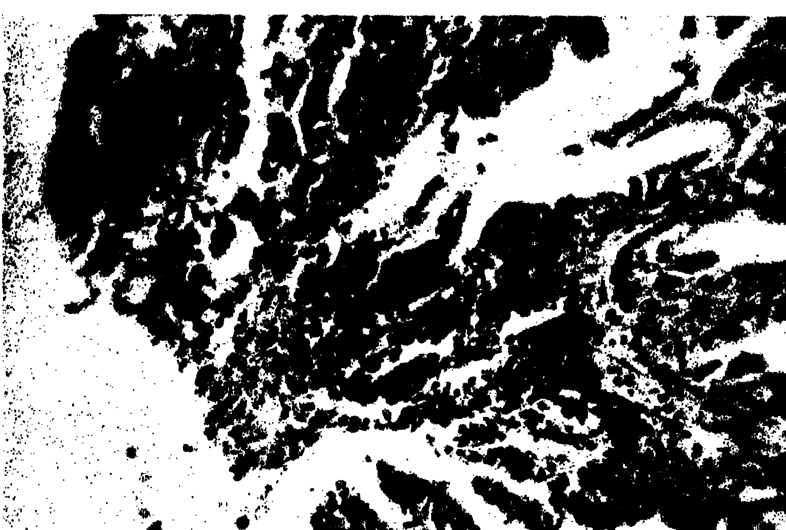

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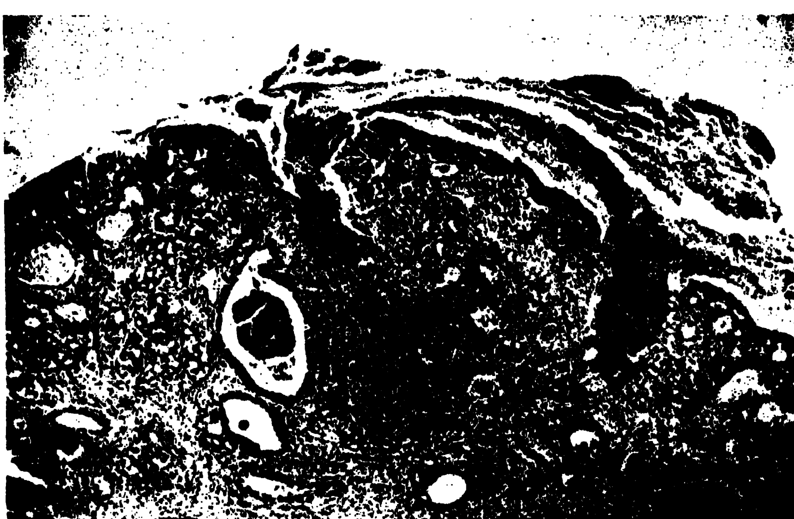

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PLATE V.

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PLATE VI.

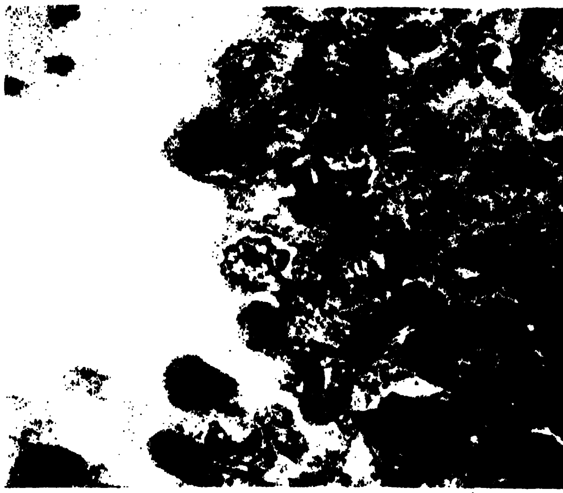

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PLATE VII.

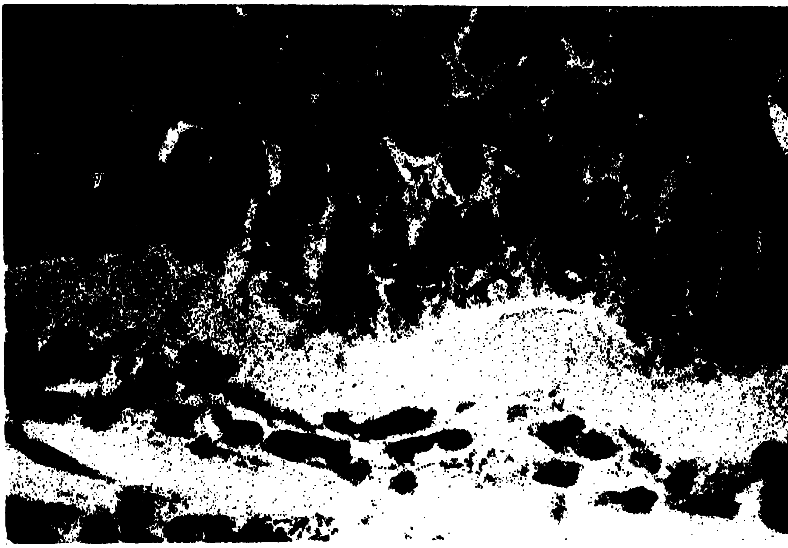

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Plate VIII.

NOTICE.

To the subscribers to the Philippine Journal of Science:

Owing to a mistake in delivery from the United States, the paper used in the JOURNAL was of necessity changed in No. 4. Such troubles arise when the consumer is at a distance of 10,000 miles from the market, and, when encountered, have no remedy. Subscribers are asked to overlook the difference in color, which will be changed as soon as possible.

THE DIRECTOR OF PRINTING.

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THE HISTOLOGY OF THE SKIN LESIONS IN VARICELLA.[1]

By E. E. TYZZER.[2]

Although varicella is generally regarded as a disease of childhood, the following report deals entirely with the skin lesions in adults. These cases were observed among adult male Filipinos, inmates of Bilibid Prison, at Manila, P. I.

Concerning the prevalence of this disease in the Islands, I could obtain but scanty information. In some localities it is recognized by the natives as a distinct disease and is called in the Tagalog "buluton tubig" or waterpox, to distinguish it from "buluton" or smallpox. In one of the provinces a native boy who showed the characteristic eruption of varicella was met, with his face pitted from a former attack of smallpox.

The occasional occurrence of varicella in adults is generally accepted, although some eminent authorities claim never to have seen a case. Thomas (15) (16) states that he never saw a case in an adult and that eruptions resembling varicella in adults indicate variola. Von Genser (6) has analyzed 29,250 cases of varicella and finds that 98.22 per cent occurred before the fifteenth year of life and 1.78 per cent between the fifteenth and sixty-first year. Race, climate and confinement in crowded prisons doubtless contribute to make adults more susceptible to this disease. In the cases dealt with in this paper the diagnosis was definitely established by inoculation experiments and by the histological study of the skin lesions as well as by the clinical features of the disease.

It was formerly a matter of controversy whether variola and varicella were etiologically identical and but different manifestations of one and the same infection. Hebra and certain others of the Vienna School have

[1] The work on which this paper is based was carried on in the Biological Laboratory of the Bureau of Government Laboratories at Manila. It was undertaken in connection with an investigation upon smallpox which Dr. W. R. Brinckerhoff and I were carrying on at the time. I am indebted to Dr. Brinckerhoff for many valuable suggestions and for the interest which he gave to this work. The opportunity for this study of varicella was afforded through the courtesy of Dr. Moulden, physician to Bilibid Prison. Funds were furnished by a grant of two Bullard Fellowships.

[2] E. E. Tyzzer, M. D., Assistant in Pathology, Harvard Medical School.

maintained that the two affections are identical, but most modern authorities are agreed that variola and varicella are two distinct diseases, produced by different infecting agents. The chief data bearing on this question, which the following cases present, are the occurrence of vaccination or smallpox previous to the varicella attack. In a study of thirty-eight cases of varicella, three had the face deeply pitted by a previous attack of smallpox, and of this trio two also bore scars from recent vaccinations. Of the remaining thirty-five cases, thirty-two bore either recent, or both old and recent vaccine scars. There is no record of vaccine scars in the other three cases, but it is not improbable that they had recently been vaccinated, for a general vaccination had been accomplished some time before these observations, on account of the occurrence of smallpox in the prison. If the two diseases are identical, as is asserted by Hebra, it is difficult to explain why the severe form as seen in *variola vera,* as well as the oft-repeated vaccinations, should not protect against so slight a form as varicella.

Varicella is recognized as an easily communicable disease, yet less is known concerning the mode of infection than is the case with smallpox. While some authorities believe that the disease is communicable from a day or so previous to the eruption up to the time of complete healing of the skin lesions, the presence of the infectious agent in the skin lesions has not conclusively been proved by experimental inoculation.

Inoculation of children with the vesicle contents of varicella has been tried in many instances but there is great discrepancy in the results obtained. Heim (7), Vetter (20) (21), Thomas (15) (16), Czarkert (2), Fleischmann (3) (4), Buchmüller (1), Smith (12), and others were unable to produce the disease by inoculation. Fleischmann from his first series of inoculations concludes that it is not possible to produce either variola or varicella by the inoculation of varicella lymph. In seven inoculations done at a later date, he obtained a general eruption in one case and a local reaction in two. Buchmüller gives a series of thirty inoculations with varicella. There was some local inflammation in children so inoculated and in one case a general varicella eruption. He regarded this as being a chance infection rather than the result of the inoculation.

Opposed to these results are those of Hesse (8), Steiner (13), d'Heilly (9), and others. Hesse made inoculations with varicella in one hundred and fifteen cases, which resulted in a local reaction in seventeen, in a general eruption in nine, and was without result in eighty-seven cases. Hesse inoculated with purulent material and dried crusts as well as with clear lymph. Both Vetter and Thomas consider Hesse's results of doubtful value: (*a*) On account of the possibility that his positive results were due to the use of smallpox lymph instead of varicella lymph; (*b*) some cases may have been due to spontaneous infection. Vetter asserts that the inoculations should be done with the perfectly clear lymph of typical varicella vesicles and should not be performed during an epidemic,

but from sporadic cases of varicella. Eimer, who has critically considered Hesse's work, concludes that only one of his cases can be accepted.

Steiner's results are, in the two cases which he gives in detail, beyond question. He inoculated two children one 4 years old and previously vaccinated, the other 2 years and not vaccinated. Neither had had varicella previous to this time. He inoculated the upper arm with perfectly clear lymph from the vesicles of a case he considered to be undoubted varicella. Four days after the inoculation there was elevation of temperature which increased, accompanied by some constitutional disturbance, up to the eighth day when a typical varicella eruption appeared. The development of the eruption together with the symptoms he describes in full. The parallelism of the two cases as regards time of onset and eruption relative to inoculation makes spontaneous infection seem improbable. He had previously inoculated eight children with varicella with positive results in six, making a total of eight successful inoculations. Of the eight cases five had been previously vaccinated, so that it seems improbable that he dealt with a mild form of variola.

The data furnished by d'Heilly's inoculations are meager. He claims to have produced an eruption in two of the cases inoculated, one after an incubation of three, the other after an incubation of seventeen days.

We see by this review that the attempts to produce varicella by inoculation show positive results in but few cases and some of these are not beyond question.

The only animal inoculations which I have found recorded are those of Freyer (5) and of Park (11). Freyer collected the fluid contents of twenty grayish-yellow, clouded vesicles, and ground it in a mortar after adding a small amount of glycerin. With this mixture he inoculated a calf without result. The calf was inoculated afterwards with vaccine lymph and reacted in a typical manner. Park inoculated two monkeys with varicella material with negative results. He points out the diagnostic value of this proceeding and cites a case in which it was used.

The only account of the histological changes in the skin lesions in varicella is that of Unna (18) (19). He takes up the changes occurring in the epidermis in the development of the vesicle and describes two types of cell degeneration which he designates "reticulating colliquation" and "ballooning" degeneration. His description of these may briefly be summarized as follows:

1. Reticulating colliquation affects chiefly the older cells of the epidermis; that is, the stratum spinosum and outer layers. The cells increase in size and in stained sections appear pale. This swelling may be followed either by a partial or total solution of the interior of the cell. The cell membrane, however, persists so that large cellular cavities are formed. If the softening of the interior of the cell is partial a coarse reticulum will be found, but if the softening is more complete the cell

will appear as a hollow chamber within which is usually a small amount of granular coagulum lying either against the nucleus or against the cell wall. By the liquefaction of the interiors of cells with the persistence of the cell walls, a reticular appearance is produced. Both nucleus and cell wall finally react to certain stains in a manner similar to fibrin.

2. Ballooning degeneration affects the cells at the base of the vesicle; that is, the Malpighian layer. The cells increase in size, lose their dense periphery and protoplasmic bridges. The nuclei become swollen, lose their network, the chromatin collects at the periphery, and direct nuclear division begins. He points out that in this case the stimulation to divide is from within the nucleus, and the centrosome and cytoplasm takes no part. Complete cell division is prevented by the early coagulation of the cytoplasm. Through this type of direct nuclear division, which takes place rapidly, large multinuclear cells are formed. Later in the process, material taking the diffuse stain appears within cells undergoing this form of degeneration, and finally both cytoplasm and nucleus stain diffusely. Thus cells presenting the ballooning as well as those presenting the reticulating types of degeneration finally undergo a "fibrinoid" transformation. In varicella lesions the ballooning degeneration is a more prominent feature than the reticulating colliquation. The formation of the vesicle is accounted for by the penetration of fluid exudate into the epidermis entering into or seperating degenerated cells and forming spaces in the tissue which enlarge and tend to coalesce to form a single chamber.

In this paper I report thirty-eight cases of varicella occurring in adult male Filipino prisoners. There had occurred in the prison about three hundred cases at the time the last observations were made and new cases were continuing to appear sporadically. Attention was first directed to them for the purpose of establishing a diagnosis. Several cases of smallpox had occurred in the prison previous to the appearance of this disease, so that it was at first important to determine whether a mild form of smallpox or a different disease was present. Later on a further study of the disease was made. This consisted of the inoculation of animals with the exudate from the skin lesions and of a study of the pathology of the skin lesions.

In two cases first seen, a few lesions were present, scattered over the surface of the body. These consisted of elevated, flat-topped pustules which possessed a central crust; this in some cases was slightly depressed. They contained a whitish, semi-fluid exudate, evidently pus. There was but little induration at the base of the pustule and the entire process was quite superficial. The exudate was collected from several of these lesions and used to inoculate one monkey and two rabbits. All inoculations were made immediately after the collection of the exudate.

Monkey No. 1.—The skin of the belly was shaved and several shallow scratches were made and inoculated with the exudate mentioned above. Four days after the inoculation the surface of the skin was elevated and slightly opaque at certain

points along the scratches. One of these elevations was excised for histological study. Six days after inoculation there were small crusts situated upon the remaining scratches. These crusts were used to inoculate the corneas of rabbit No. 4. Nine days after inoculation, three small papules 2 mm. in diameter were present in the vicinity of the inoculation scratches. One such papule was excised for histological study.

About a week subsequent to the last observation the monkey was inoculated with lymph from a case of variola in man and typical variola lesions developed.

Stained paraffin sections of the skin excised four days after inoculation show the epidermis thickened over a small area, which is surmounted by a minute crust. There is no destructive process, only such a condition as might occur in the repair of an ordinary scratch. The papule excised nine days after inoculation shows a loss of epidermis over a minute area which is covered by a very delicate crust. (Such lesions were later found quite frequently in instances where the animal's belly was shaved and were regarded as minute abrasions from scratching.)

Rabbit No. 2.—The eyes were anæsthetized with two per cent cocaine solution and the exudate from the human skin lesions rubbed into shallow incisions made upon the cornea. The corneas appeared smooth after twenty-four hours and, as no process could be detected in the daily observations for a week following the inoculation, the animal was not killed.

Rabbit No. 3.—Both corneas were inoculated with the same exudate and in a similar manner as in rabbit No. 2. The corneas in this case healed rapidly and no process developed subsequently. Animal not killed.

Rabbit No. 4.—Both corneas were inoculated with the crusts obtained from the inoculation of Monkey No. 1. The corneas became nearly smooth in from twenty-four to forty-eight hours. The animal was killed three days after inoculation. In sections of the cornea there appeared a slight defect in the epithelium at the point inoculated, but no pathological process beyond that which might follow an ordinary injury was present.

A number of cases subsequently appearing among the prisoners were seen at what was evidently an earlier stage in the development of the eruption. These presented full hemispherical or oval vesicles which were easily ruptured and which contained clear fluid. Certain other cases seen with these presented lesions with a central crust surrounded by a vesicular zone containing either cloudy fluid, or whitish semi-fluid pus. Exudate was collected from both sorts of lesions and used to inoculate two monkeys.

Monkey No. 5.—The belly was shaved and the clear vesicle contents mentioned in the text was rubbed gently into superficial incisions made at intervals upon this area of the skin.

Another portion of the clear fluid was blown into the nostril of the monkey. This animal developed no skin lesion although under observations for several weeks.

Monkey No. 6.—The skin of the belly was inoculated in a similar manner with the semi-fluid pus from the cases above mentioned. No lesions developed from this inoculation.

The negative character of these inoculations indicates clearly that the disease is distinct from smallpox. The result was the same whether the pus or the clear fluid was used. The virus of smallpox, even when collected from the mildest cases, produces a typical process whether

inoculated on the skin of the monkey or the cornea of the rabbit. Furthermore, the clinical aspect of the disease, now that new cases were appearing, made the diagnosis of varicella already indicated by the inoculation results, certain.

Before passing on to the description of the varicella lesions it may be well in this connection to give the results of some further experimental inoculations made at a later date. The fluid contents of vesicles was collected from three typical cases of varicella. In two cases the vesicles were perfectly clear, in the other one many vesicles were slightly clouded. The fluid collected was used to inoculate a series of seven rabbits. The mode of inoculation was first to anæsthetize the eye with a few drops of 2 per cent cocaine solution so that it could be pressed gently from its socket and kept immobile while inoculating. In most instances two or three shallow parallel incisions were made across each cornea just deep enough to penetrate the epithelium. The fluid to be inoculated was then placed upon the surface and rubbed gently into the incisions. The rabbits were killed at twenty-four hour intervals after from one to six days and the corneas fixed in Zenker's fluid. The seventh animal was allowed to live for an indefinite period.

Rabbit No. 37.—Both corneas were inoculated with cloudy fluid from vesicles of case No. 32. The animal was killed twenty-four hours after inoculation; the corneal surface was nearly smooth.

Rabbit No. 38.—Killed seventy-two hours after inoculation with vesicle from case No. 32. The corneal surface appeared smooth and without defect in epithelium. There was no conjunctivitis.

Rabbit No. 39.—The corneas were inoculated with clear fluid from vesicles of cases Nos. 34 and 35. Killed forty-eight hours after inoculation. The right eye showed marked conjunctivitis, and there were several minute whitish spots in the surface of the cornea.

Rabbit No. 40.—The corneas were inoculated with clear lymph from cases Nos. 34 and 35. Killed four days after inoculation. Corneal surface nearly smooth.

Rabbit No. 41.—Corneas inoculated with clear lymph from cases Nos. 34 and 35. Killed five days after inoculation. Corneas nearly smooth.

Rabbit No. 42.—Corneas inoculated with clear fluid from cases Nos. 34 and 35. Killed six days afterwards. Corneal surface smooth. No inflammation.

Rabbit No. 43.—Corneas inoculated with clear vesicle fluid from cases Nos. 34 and 35. This animal was not killed. The incisions of the corneal surface healed rapidly after the manner of aseptic wounds and no apparent process developed during the fortnight in which the animal was kept under observation.

In the histological study of the corneas of this series of rabbits nothing was revealed, with the exception of one case, beyond the usual process of repair which follows an uninfected wound. In rabbit No. 39 one cornea showed a marked inflammatory process. Microscopically there were collections of bacilli growing in the depth of the incision and below the epithelium. The epithelium was thin and many cells were degenerated. The adjacent corneal tissue was infiltrated with leucocytes.

In no case did the gross or the histological appearances of the inoculated cornea suggest a process beyond that of repair, except one case in which there was considerable inflammation. In the latter case the inflammatory

changes were evidently caused by masses of bacteria growing in the tissue of the cornea.

Grouping this latter series of inoculations with those previously done, there were in all nine rabbits inoculated upon the cornea with the vesicle contents of varicella. Of these, five were inoculated with the clear, fluid exudate, two with the clouded fluid, and two with the semi-fluid pus; the latter material came from cases which, although at first considered doubtful, were unquestionably varicella as was proved by further observations. In no case was the inoculation followed by any specific process. Three monkeys were inoculated upon the skin, one with clear fluid, and two with semi-fluid pus from varicella lesions; all gave negative results. Thus, up to the present time it has not been possible to produce any form of the disease in animals by the inoculation of vesicle contents of varicella. Although the data furnished by the inoculation of three monkeys is meager, yet the results stand in marked contrast to those obtained through the inoculation of monkeys with variola virus.

Further study was directed to the pathological process concerned in the varicella eruption. As previously stated a study was made of thirty-eight cases and attention was paid to the development of the eruption and the general condition of the patient. A more critical study with daily observations was made of eleven of the more favorable cases. These were selected because they were seen soon after the first appearance of the eruption and were uncomplicated by the appearance of skin lesions other than varicella.

Concerning the clinical features of the disease there was nothing presented more than may be found in the text-book descriptions. The cases were usually discovered within twenty-four hours after the appearance of the eruption. In some of the severe cases there was considerable fever and constitutional disturbance, but in the majority there was no complaint of feeling ill. All degrees of severity were represented from mild cases in which there were not over a dozen lesions in the entire course of the disease to severe ones in which the lesions were thickly sprinkled over the entire surface of the body. Lesions were present in either the palms of the hands or the soles of the feet in twenty-seven of the thirty-eight cases. The following case is given in order to convey an idea of the character of the disease.

Case No. 8.—Adult male Filipino seen on June 2. Eruption appeared within last twenty-four hours. Feels rather ill. He presents two good scars from former vaccination. An eruption scattered over trunk, arms, and legs, with only three lesions on face, consists of vesicles and small red macules. The vesicles are oval or hemispherical, superficial, tense, contain clear fluid, and are situated upon a reddened area of skin. The larger vesicles measure about 5 mm., the smaller ones are barely discernible. They are easily ruptured. The macules consist of small, reddened areas of the skin, in some cases slightly elevated but barely palpable. In some such areas there is a minute central vesicle, in others the center is a rough, slightly granular elevation. Certain lesions were marked for future observation.

June 3.—Several of the vesicles observed yesterday are now slightly clouded. Many macules have developed vesicles and certain vesicles have increased in size. Lesions numerous on back.

June 4.—A great number of the vesicles have become clouded and are no longer tense. Few new lesions have developed except a number on the hands. Soft palate presents three grayish erosions. Several lesions on the face have been ruptured. One present in the scalp.

Beneath a small collodion dressing on the chest is a group of clear vesicles following the outline of the collodion.

June 6.—Many of the vesicles have dried up and are represented by thin crusts. Others persist as flaccid vessicles containing cloudy whitish fluid. No new lesions have appeared since June 4. One lesion on trunk presents a depressed thin central crust and an elevated pustular edge.

June 7.—Only three unruptured vesicles left on body. Many have disappeared or are represented by pale spots in the skin. In some cases where the remains of the vesicle have been removed there is a small, round, red area representing a defect in the epidermis. Around this is a pale, translucent zone, representing the new epithelium growing in to replace that destroyed in the lesion.

June 8.—Healing has proceeded still further. Many of the crusts have been removed leaving white, rounded areas.

Lesions were excised from the eleven cases at various stages from the earliest reddening of the skin through their entire development until healing was well advanced. The technique of incision is as follows: A fairly large-sized scalpel, which must necessarily be sharp, is all that is absolutely necessary, although a pair of mouse-tooth forceps can be used to advantage in certain instances. The fixing fluid should be already prepared and at hand. The skin is first washed with alcohol and sponged lightly with a piece of gauze or absorbent cotton to render it clean. The skin adjoining the lesion is pinched up between the thumb and forefinger so that the lesion is situated at the summit of the elevation. By holding the knife nearly flat with the surface of the skin, one cuts with a sawing motion beneath the lesion which, when removed, should be placed immediately in the fixing fluid. In certain cases when the skin about the lesion is not sufficiently loose to be pinched up by the fingers, elevation may be accomplished by seizing the skin near the lesion with the mouse-tooth forceps and then exicising. It is essential to work with a very sharp knife, otherwise the lesion is liable to become mutilated and the patient caused unnecessary pain. I would recommend this method for general purposes of diagnosis of superficial skin lesions, for the following reasons: It causes a very superficial injury amounting to scarcely more than a slight abrasion, which under ordinary conditions heals rapidly. By the thinness of the slice of tissue almost perfect fixation is obtained. By this method, in which the lesion itself is neither seized with forceps nor manipulated in any way, the topographical relations are undisturbed. The only objection to the method is that it can not be done without a certain degree of pain, for the injection of a solution of cocaine so distends the tissue that it may be impossible to acquire any conception of the original process which was present. The method of freezing the

skin before excision, sometimes used, is very objectionable. The blood is destroyed and the tissue cells are so altered that the subsequent staining is affected.

The tissue was generally fixed in Zenker's fluid. Lesions were excised twelve hours, one, two, three, four, five, six, and seven days after the eruption was first noted by the patient. On account of the appearance of successive crops of vesicles, it was often possible to obtain early lesions late in the disease so that the time interval, dated from the first appearance of the eruption, forms no index as to the stage of the process present. In order to acquire a more accurate knowledge of the development of the lesions, certain ones were ringed with indelible ink at their first appearance and the subsequent changes noted.

The following description of the process is based on a study of a series of lesions of the type which appears most constant, beginning with the earliest recognizable stage and taking up successive stages until repair is well advanced. In such a series of lesions the process is found to consist of changes occurring simultaneously in both epidermis and corium. However, for purposes of description, first the changes affecting the epidermis and subsequently the changes affecting the corium will be discussed. The same order will be followed in the description of the later changes which result in the repair of the skin lesions. Following this the more unusual forms of the lesion will be described separately.

The earliest lesions are obtained by the excision of red spots which precede the formation of vesicles. The first change in the epithelial cells consists of a swelling both of the cytoplasm and of the nuclei so that the epidermis is considerably thickened over a small area. (Pl. I, fig. 1.) The process in the epidermis is ofeten not confined to a single center. It is common to find multiple distinct centers separated by normal epithelium, and multiple centers which merge one into another forming a conglomerate lesion. (Pl. I, fig. 2.) With the swelling of the cells the cytoplasm stains more faintly and becomes distinctly reticulated. The nucleus is also distended so that it appears large and hollow. These nuclei almost invariably contain one or several masses of eosin-staining material. The nuclear network and the chromatin masses are retracted away from this material so that it appears to occupy a clear space within the nucleus. There are constantly present in addition to the nuclear inclusions, small deeply staining, rounded masses, situated on the outer surface of the nuclear membrane, in the cytoplasm, or between the epithelial cells. A discussion of the cell-inclusions associated with this process will be taken up in a subsequent paragraph.

Foreign cells occur here and there in the affected areas of the epidermis. From the study of the changes in the underlying corium, it seems probable that these are cells of endothelial origin which have migrated into the epidermis. In many instance these extraneous cells are found in portions of the epidermis where there are but few cells deviating from

the normal type. Thus very minute lesions of the epidermis are found in which no more than three or four cells show marked change. (Pl. IV, fig. 21.) Some of the cells which have wandered into the epidermis are in a state of good preservation and these often contain eosin-staining nuclear inclusions similar to those which occur in the epidermal cells. Others present various degrees of degeneration and many have become disintegrated. The small masses resulting from their disintegration may consist of either nuclear or cytoplasmic material, or both, and are readily distinguished.

The cell changes in the epidermis following the initial swelling are progressive. Two types may be distinguished. In that termed by Unna "reticulating colliquation," either the interior of the cell liquefies or a space is formed within it, while the periphery persists as a dense membrane. Within such hollow cells the nuclei are often collapsed, a condition possibly produced by changes in the surrounding cell fluid. This form of degeneration is not so prominent, in the varicella lesion, as that next to be described.

The term "ballooning degeneration" is given by Unna to certain changes resulting in the formation of large multinucleated cells with soft plastic cytoplasm. The first change is an increase in the size of the cell while the nucleus becomes large and vesicular; the nucleus then divides by direct division, and this process is rapidly repeated until a large number of nuclei are produced within the cell. The cytoplasm is basophilic, and in it a distinct reticulum appears. The limiting membrane and protoplasmic bridges of the cell become less distinct and after a time disappear. Such cells attain enormous dimensions. The nuclei are usually grouped in the centre of the cell, often so closely packed together that the opposing surfaces are flattened one against the other. These nuclei appear as hollow sacs with one or several masses of chromatin peripherally situated, and with an eosin-staining inclusion in the interior of each nucleus. There is scarcely any indication of a nuclear network. In the presence of such a degree of amitotic division, it is nevertheless difficult to find nuclei caught in the act of division. The chromatin in some instances projects as a septum nearly across the nucleus. The dividing split passes directly through this projecting chromatin so that an approximately equal portion goes to each of the resulting nuclei. Although this would seem, from the common occurrence of nuclei flattened against one another, to be the common form of direct division, yet it can not be considered to occur constantly in this process. Certain nuclei are dumb-bell shaped and the two portions are joined by a very thin filament. Some are lobulated and are apparently in the process of constricting off small portions of their substance which form accessory nuclei of varying size. This latter form of nuclear division is seen in a greater degree in the migrating cells and in the endothelium lining the lymphatics of the corium, than in the epithelium.

With the progressive cell changes in the epidermis, exudation becomes an important factor in the typical process. Fluid finds its way into the degenerated epithelium and separates the degenerating cells so as to form spaces filled with fluid. In this manner the formation of the vesicle is begun. That the exudate sometimes breaks into hollow cells is proved by the occasional presence of free nuclei in the smaller chambers. The exudate may at this early stage appear perfectly clear or it may contain a small amount of fibrin either in the form of a reticulum or of a granular precipitate.

The continuous escape of the exudate into the epidermis rapidly dilates the smaller chambers into a large vesicle. In case the lesion has developed from multiple centers, the various chambers are separated by septa and trabeculæ which are prone to rupture on account of the rapidity of exudation. The chamber begins to form in the middle layer of the epidermis so that a portion of the stratum spinosum is lifted up with the horny layer to form the roof, while the floor of the vesicle is at this stage wholly or partially covered with epithelial cells.

At the sides of the vesicle and in the trabeculæ, the cells become elongated and stretched through the pressure of the exudate. With the cells thus stretched, the epithelial fibers of Herxheimer become prominent. It seems evident that there is an actual production of fibers coincident with the swelling and stretching of the cells. They appear as straight fibers, with tapering ends, extending lengthwise over the surface of the epithelial cells. In some cases the cells become torn from their original attachment and the fibers then released take on a spiral or crinkly form. (Pl. II, fig. 10.) They vary much in thickness and where cells lie free in the vesicle contents they often extend directly into the cell and terminate against the nucleus.

Lying free in the vesicle contents or in groups over the floor of the vesicle and along the trabeculæ are epithelial cells showing various forms and stages of degeneration, prominent among which are the large multinucleated cells resulting from the so-called "ballooning degeneration." These cells now present further degenerative changes. The protoplasm is often condensed into a membrane about the nucleus or the group of nuclei. External to this membrane the cytoplasm is very soft in appearance, being composed of a delicate reticulum. In this delicate portion, deeply stained granules or masses appear. On the surface of such cells there are sometimes globular or elongated masses staining faint blue, which radiate from the cell like the petals of a flower. In many cases the uniformity of these bodies and their arrangement is suggestive of some sort of an organism. After studying a number of cells of this type, the observer is convinced that the masses are pseudopodia-like projections from the soft protoplasm or portions of it which have been constricted off. In some cells the so-called "acidophilic" or "fibrinoid" degeneration has become marked and the entire protoplasm appears

hyaline and deeply stained with eosin. The hyaline transformation makes its first appearance about the nuclei either in the form of a dense membrane or as irregular masses.

The base of the vesicle in which there is an active exudation is almost invariably concave. The papillæ are either totally obliterated or distorted through lateral distension. The epithelium in many cases persists between these papillæ for some time after it has been destroyed over the more exposed portions. The vesicles are in some instances multilocular but the average one is unilocular and stands in marked contrast to the type of lesion found in smallpox. The common involvement of hair follicles and sweat ducts in the typical rounded vesicles indicates clearly that these structures acting alone do not produce umbilication.

After the vesicles have become fully formed, other changes occur. To the naked eye the vesicles are flaccid and no longer tense, and the fluid is clouded. This condition may be seen three or four days after the first appearance of the eruption or it may even be present earlier. The evaporation and absorption are now more rapid than the out-pouring of the exudate. The fluid within the vesicle contains more fibrin than in the early stages and phagocytes which are attracted by the dead epithelial cells are beginning to appear. The roof of the vesicle, the trabeculæ, and the cells of its lateral wall now take a diffuse stain and are apparently dead. The trabeculæ are ruptured in almost every instance and their remains are to be found projecting downward from the roof of the vesicle. The former pressure of exudate has in many instances broken peripherally beyond the boundary of the original lesion so that irregular spaces are found. The large, rounded epithelial cells lying loose on the floor of the vesicle show various degrees of hyaline changes. Some appear dense and hyaline, others are stained but faintly. The nucleus is either no longer apparent or is represented by irregular, deeply stained masses. Probably all the epidermis involved in the lesion is eventually destroyed.

In some instances a portion of the epithelium which shows none of the cell changes that are characteristic of the process, is invaded by numbers of phagocytic cells. It is possible that such infiltration of a small focus of the epidermis represents the site of a minute process in which the initial cells changes have been obscured in the infiltration which has followed. The phagocytic cells invade the normal epidermis and are found in varying number far from any well defined vesicle. Cause for their presence is not to be found in any injury to the epidermis.

In all the early lesions a pathological process is constantly found in the corium. The first change noted is the presence of eosin-staining inclusions situated usually in the nucleus, more rarely in the cytoplasm of cells. In what may be considered a very early stage in the process the eosin-staining inclusions are limited to the endothelial cells lining the small blood vessels. Later, when the process is well advanced, similar inclusions occur in a large proportion of the cells in the corium under-

lying the vesicle and in almost every type of cell here represented. These inclusions are associated with cell changes similar to those already described in the affected epidermis. The nuclei become distended and tend to divide by amitosis without subsequent division of the cytoplasm. Therefore, early changes of this type are found constantly in the corium as well as in the epidermis of the varicella lesion. It seems probable that the changes in the corium antedate those in the epidermis, for they are always present and often well marked in the earliest and most minute lesions.

Further changes occur in the corium at first appearing chiefly in and around the blood vessels. The process in more advanced in the neighborhood of the epidermal foci, but slight changes occur over much larger areas. The endothelial cells lining the small blood vessels present various degrees of proliferation as shown by the presence of numerous mitotic figures. Immediately around the vessels are collections of cells of which the most numerous have a large vesicular nucleus and more or less basophilic cytoplasm. It seems probable that these cells are of endothelial origin, arising from endothelial cells in the lymph spaces and vessels adjoining the blood vessels. In favor of this view is the fact that numerous mitotic figures occur in the cells lining these lymph vessels. Later in the process these endothelial cells become phagocytic. Mingled with these cells are a few lymphoid cells, and an occasional eosinophile, but the polymorphonuclear leucocyte is not represented.

In addition to the early cell changes already described, more advanced degeneration is found in cells in the vicinity of the affected vessels. The degeneration is not general; only scattered cells are affected. In some instances an entire cell appears hyaline and stains intensely. In other cells a form of nuclear fragmentation has taken place and the chromatin is scattered throughout the cell in the form of minute deeply stained globules. Portions of disintegrating cells are found occasionally. The destruction is often so far advanced that it is impossible to determine, in single instances, the type of cell affected, but it seems certain that the cells previously described as of probable endothelial origin are frequently destroyed. Changes in the corium like those already described are found in all early lesions.

In lesions taken at a later stage, during the formation of the vesicle in the epidermis, a more extensive process is found in the corium. The endothelial cells, although still most numerous about the blood vessels, are not confined to this locality. Large numbers are found scattered through the corium beneath the affected epidermis; however, the number of these cells migrating in the tissue varies considerably in different lesions.

It is not uncommon to find the capillaries and small vessels dilated just beneath the vesicle. The lymphatics also are dilated in some instances but, with the exception of those which occur immediately about

the blood vessels of the lesion, there seems to be no noteworthy change affecting them.

In some lesions a great proportion of the cells in the corium below the vesicle present those peculiar changes characterized by the presence of eosin-staining nuclear inclusions and direct nuclear division. The cells are tranformed to such a degree that it is often impossible to decide to what type certain ones belong, but it is evident that nearly all varieties of cells are affected including endothelial cells, both *in situ* upon the vessel wall and free in the tissue, connective tissue cells, pigment cells, and eosinophiles. The nuclei become swollen, irregular, and lobulated, and segment irregularly into smaller nuclei, often of unequal size. The cytoplasm is increased to such an extent that cells of relatively enormous size are produced. When such large cells are of connective tissue origin, the cytoplasm presents irregular branching processes. Occluded blood vessels were found in several instances. The occlusion was due to the presence of large multinucleated cells within the vessel. In another instance a group of large multinucleated cells extended through sixteen sections (eighty micromillimeters) of the tissue in close relation to a blood vessel. It would seem that large multinucleated cells occasionally develop from the endothelium of the blood vessels and lymphatics involved in the lesion.

Later in the process phagocytosis becomes a prominent feature. The endothelial cells are found in varying numbers in the cavity of the vesicle where they are engaged in devouring the remains of epithelial cells. That portion of the corium which forms the floor of the vesicle is infiltrated with these phagocytic endothelial cells. Phagocytosis is found in a greater or less degree throughout the corium of the lesion. At this stage small numbers of polymorphonuclear leucocytes are present in the corium. On the other hand, if bacteria have gained entrance, polymorphonuclear leucocytes are found infiltrating the tissues in great numbers. Their appearance seems to be of the nature of a secondary phenomenon rather than an essential part of the process.

In the majority of lesions the active process ends soon after the destruction of the involved portion of the epidermis is completed. Many of the resulting necrotic epithelial cells appear either as dense hyaline or as faintly stained masses both free in the exudate and along the floor of the vesicle. Phagocytic cells are present in varying numbers but there is usually a large number present in the cavity of vesicles of four days duration. In the uncomplicated process, polynuclear leucocytes are relatively rare even in the older vesicles. When the destructive process is at an end, the demarcation between the living and the necrotic epithelium becomes distinct. The former grows inward over the floor of the vesicle, following the surface of the papillæ. This rapidly growing epidermis presents the same characteristics as that growing over any defect. The absence of pigment in the newly formed epidermis accounts

for the whiteness of recently healed varicella lesions in Filipinos. The vesicle may persist as such until the surface beneath is completely healed, or it may be ruptured and form a thin crust on drying.

In the corium, the migrating cells gradually become reduced in numbers, the infiltration about the blood vessels is diminished, and with the repair of the defect in the epidermis the underlying tissue also returns to its normal state.

In addition to the typical lesions upon which the description of the process has been based, certain other atypical lesions occur.

Vesicle within vesicle.—In a certain percentage of the lesions there is a secondary extension of the lesion. After the vesicle is apparently well developed, it still remains tense and when seen on the following day, an increase in size is noted and the outline of the former vesicle is seen within the large lesion. The original vesicle is clouded and opaque, while the newly formed one is clear. This condition is brought about by the leakage of fluid from the cavity of the original vesicle, into the horny layer which it splits. (Pl. III, fig. 15.) It seems probable that the larger blebs and bullæ sometimes met with in these cases are formed in this way, as they appear to be very superficial.

Dry or abortive lesions.—In a small number of lesions there appears a marked localized degeneration of the epithelial cells unaccompanied by any appreciable exudation. These lesions appearing as reddened, slightly elevated spots in the gross show microscopically a portion of the epidermis wholly necrotic, the cells of which are disassociated, stain red, and are hyaline in appearance like those in the advance vesicle. Reticulating colliquation is not represented. Such lesions are simply lacking in fluid exudate. (Pl. III, fig. 16.) This condition is difficult to explain, especially when cases occur in which a majority of the lesions develop in this way. A case illustrating this condition is as follows:

Case No. 28, June 8, 1904.—A Filipino, young adult, having two old vaccination scars. The eruption has appeared within the last twenty-four hours, and is scanty. It is papular on the face and mixed with acne. Several clear vesicles, 5–6 millimeters in diameter in groin, few papules on legs. A vesicle 5 millimeters on anterior thorax, one 2 millimeters back of the shoulder, and a few small vesicles on arms and forearms.

June 9, 1904.—Eruption has increased and is scattered over the entire body. It consists of small reddened spots, the majority of which have a slightly elevated granular surface. None on hands or wrists. Face, rough and papular. Mouth negative.

June 10, 1904.—The eruption is now profuse and simulates an early smallpox eruption. It consists of small red papules two to four millimeters across, with granular tops. Some papules have at their summit a tiny vesicle in the center of a slightly roughened surface. A few clear, hemispherical vesicles have developed. Lesions are profuse on face and trunk, scattered over arms and legs, none on palms.

June 11, 1904.—Very few papules have become vesicular. Many lesions have faded out and practically disappeared. Following this date no new vesicles appeared and those present healed rapidly.

Lesions in the hair follicles.—It is common for hair follicles to be involved in the vesicle of varicella, but the process is at times so limited that it does not reach the surface of the skin. This occurred in two lesions excised. In one was an early process affecting the sebaceous gland and hair sheath. (Pl. II, fig. 8.) In this lesion were all the forms of cell degeneration already described. There was but slight infiltration with wandering endothelial cells and no vesicle formation. In another lesion similarly situated the sebaceous gland and the lower portion of the hair sheath were infiltrated with endothelial cells and there was a small chamber containing fluid. In places the cell degeneration could be made out to such an extent as to identify the process as varicella.

Lesions about the ducts of sweat glands.—These ducts are involved in a great many lesions. They may be seen at points along the base of the vesicle (Pl. III, fig. 15), or portions may persist within the chamber of the vesicle. Some of the minute foci are found about the epidermal portion of these ducts. (Pl. I, fig. 4.) In such instances the cells lining the duct are unaffected while the process is confined to the epidermis which surrounds it. In no case was the characteristic initial change of the process found in these ducts.

Vesicle infected by bacteria.—A certain number of lesions do not heal immediately after the full development of the vesicle. The contents becomes white and opaque and the vesicle is topped with a central crust. Vesicles presenting such an appearance have probably been ruptured, so that bacteria have gained access, although it is possible that bacterial infection may in some instances occur without the vesicle being ruptured. Such lesions exend slowly, the central crust becomes larger and surrounded by a pustular ring. The depression of the central crust as compared with the elevated pustular periphery furnishes a form of umbilication. It is plain that this lesion is secondary to varicella and has nothing to do with the active process. The umbilication is therefore of a false variety. On account of the inadvisability of excising such lesions, only one small lesion of this character was obtained. In this the pustule contents was composed almost wholly of polymorphonuclear leucocytes. The corium forming the base of the pustule was infiltrated throughout with these leucocytes and there was considerable destruction of tissue in the papillary layer. It seems probable that the permanent scars, which are not an uncommon sequence of varicella, result from lesions of this type. In the milder, uncomplicated lesions, in which the papillary layer is not preceptibly damaged, the regeneration of the destroyed epithelium constitutes perfect repair and no scar is produced.

The earliest changes in the varicella process are associated with cytoplasmic and nuclear inclusions which are found in epidermal cells, endothelial cells *in situ* within the blood vessels and lymphatics as well as those which are found migrating in the tissue, connective-tissue cells, pigment cells, and eosinophiles.

The nuclear inclusions appear at first as minute eosin-staining granules about 1 micromillimeter in diameter, situated in the nuclear sap. The nucleus may contain one or several. A clear space is formed about each nuclear inclusion and the nuclear network recedes from it. The inclusions rapidly increase in size and stain more intensely. They are most often rounded, but may be elongated or irregular. In some instances the nucleus is affected only about the inclusion, at which point it is greatly distended while the remainder appears normal. (Pl. III, fig. 18.) Very irregular and lobulated nuclei are thus produced in the cells of the corium. In the epithelial cells the whole nucleus becomes distended, the chromatin and nuclear network retracts to the periphery, leaving the interior about the inclusion clear. Direct nuclear division follows these changes, and each of the resulting nuclei usually contains a characteristic inclusion. These inclusions may attain a diameter of 6 microns when rounded and 9 microns when stretched out in elongated form. Some appear dense and homogeneous, while others are granular in character. Indefinite, deeply stained points are occasionally to be made out in them. In some instances minute granules of similar substance are grouped about a large inclusion. In one case in which the knife had passed through a sebaceous gland in excising the lesion, the nuclei were ruptured and the inclusions here showed characteristics not apparent elsewhere. (Pl. II, fig. 12, and Pl. IV, fig. 25.) They were evidently composed of a plastic substance as they had a sharp though irregular outline with rounded projections suggestive of a flowing motion. They consist of a sharply contoured body, delicately reticulated in structure. Certain ones showed a central, deep-staining granule. A certain number of nuclear inclusions are found which either are attached to the nuclear membrane or are protruding through it. They show a marked constriction where they pass through the membrane. A large proportion of these inclusions present the form of an irregular, reticulated mass without definite contour. Although these inclusions take the red in preference to the nuclear stain, they stain deeply by other methods so that they would not readily be distinguished from chromatin.

The cytoplasmic inclusions stain a dense purple by the eosin and methylene blue method, and measure from 1 to 4 micromillimeters in diameter. They are often found lying against the outer surface of the nuclear membrane, but may also occur between cells free in the connective tissue or in any position in the cytoplasm. They are constantly present in moderate numbers in the epidermis and in the cells of the corium of the early lesions, but are usually less numerous than the nuclear inclusions. They always possess a sharp contour, are most often rounded, but are occasionally irregular, and have usually a deeply stained central point. The occurrence of nuclear inclusions protruding through the nuclear membrane suggests a possible method of origin for the cyto-

plasmic bodies. They do not appear to be related to the fragments of disintegrated cells which are of common occurrence in the lesion.

It is difficult to reach a conclusion concerning the nature of these bodies. They are associated with the earliest changes and constitute one of the constant characteristics of the process. In a small portion of the inclusions there is some evidence of internal structure, but this is so indefinite that it carries but little weight as to their nature. A large proportion of the nuclear inclusions are so structureless and irregular that it is difficult to believe that they are living organisms. In spite of the great rapidity of the process it has not been found possible to demonstrate a multiplicative process in these bodies.

In the fresh preparation they appear homogeneous and a large number of inclusions were watched for several hours under the microscope during which time they did not change in outline. Furthermore, eosin-staining material is found within the nuclei in various other processes and in normal tissues. The writer is, however, familiar with no condition which presents inclusions closely simulating those found in varicella lesions.

As the earliest nuclear inclusions appear as red-staining granules in the nuclear sap, it is possible that they represent an increase of a substance, such as linin, present in normal nuclei. It seems to be certain that elements, readily overlooked in normal tissue, may become prominent in pathological conditions. Such an example is found in these same varicella lesions in which the epithelial fibers of Herxheimer, which are occasionally met with in normal epidermis, become increased to such an extent that they form one of the most striking features of the process. (Pl. II, fig. 10.) In this example, however, the elements in question can be traced in their transition from normal structures and are always confined to the epidermis. The nuclear and cytoplasmic inclusions, on the other hand, present a constant morphology without transitional stages connecting them with normal structures and are not confined to cells of any one tissue or type of tissue.

Another plausible hypothesis is that the inclusions represent the formation in a pathological condition of some substance not found in the normal nucleus. The reaction of the nucleus accompanying the presence of the inclusion is peculiar in that only that part about the inclusion is affected, while the remainder of the nuclear structure appears normal. If the inclusions are the result of a changed metabolism, one would expect to find a more general change. A fact to be emphasized concerning the nature of these inclusions is that they are confined to small foci, even to individual cells in the epidermis and corium. There is not sufficient evidence at hand to interpret either the nuclear or cytoplasmic inclusions and it seems preferable to suspend one's judgment until new facts are acquired bearing on this question. They are described because they form a constant and prominent feature in the varicella process.

The failure to demonstrate that the disease can be communicated by inoculation with the contents of the vesicles is against the idea that the infectious agent is contained in them. This evidence is not absolute and moreover a necessary corrollary of such experiments would be the excision and study of the area inoculated. It might well be that a slight but characteristic lesion may be produced which would not be apparent on macroscopic examination and which would not lead to the production of an exanthem. If we regarded the production of the entire disease as necessary to constitute infection, the infectiousness of smallpox in the inoculation of the calf or rabbit would be denied. On the other hand the lesions in the disease are so cellular in character and the cellular changes in their entirety so characteristic that it is hardly conceivable that the process can be produced by anything other than some agent which enters into and affects the single epithelial cells.

That the infectious agent is disseminated by the blood is rendered probable by the common occurrence in early lesions, of a process of wider distribution in the corium than is found in the epidermis and by the occurrence of isolated lesions of blood vessels far from any epidermal involvement. It seems reasonably certain that the process in the corium antedates that in the epidermis.

Certain facts brought out in this investigation may be given a practical application in the matter of diagnosis. During every epidemic of smallpox there occur cases in which there is great difficulty in diagnosis. One needs but to scan the literature to acquire a conception of the number of these difficult cases. Stelwagon (14) quotes an analysis of thirty-eight cases of error in diagnosis of smallpox, of which seventeen turned out to be varicella. There has been some dispute in regard to the nature of a widespread epidemic reported from Trinidad, whether the cases were all varicella or whether some were those of a mild form of smallpox. There is no doubt that many cases are passed over without their true nature being recognized. One can not depend absolutely on the gross appearances of the lesions or on the distribution.

It seems a matter of considerable importance to be able to distinguish from true varicella certain cases of smallpox resembling varicella, which, although mild, give rise to severe variola in other individuals.

Certain methods concerning the diagnosis of smallpox have already been suggested. The inoculation of monkeys as recommended by Park (11) is not always practicable in that monkeys are expensive and not always obtainable.

Howard (10) suggests in addition to animal inoculation, the study of sections of excised skin. The diagnosis is based upon the character of the process and the presence of *C. variolæ.*

Thompson (17) has applied a rapid method for embedding the excised lesions, by which he is enabled to obtain sections ready for study within three hours after excision.

The following methods are given, since they may be found of value in the diagnosis of cases in which there is a question of smallpox or varicella:

1. The contents of early, clear vesicles of such a case may be examined fresh under the microscope. The presence of large multinucleated cells is consistent with varicella and against smallpox. This test seems quite reliable and may be applied at the bedside.

2. The excision of a typical lesion for histological examination may be resorted to. In the smallpox lesion the presence of *C. variolæ* will furnish the diagnosis. In varicella the type of the vesicle, the ballooning degeneration and the constant presence of the described intranuclear and cytoplasmic inclusions will render certain the diagnosis. As will readily be inferred, the simple presence of definite cytoplasmic or nuclear inclusions within the epithelial cells is not sufficient to establish the diagnosis of smallpox, for both nuclear and cytoplasmic inclusions are also found in varicella. If the presence of these bodies is to be taken into account in the diagnosis one must possess a certain degree of familiarity with them, in one or both of the diseases in question, in order to determine their specificity.

3. A slower but very reliable means of diagnosis is the inoculation of a rabbit's cornea with fluid from the lesions. With smallpox lymph a variolous keratitis is produced which is evident in the roughening of the corneal surface in from twenty-four to forty-eight hours after inoculation. The inoculation of varicella lymph gives no process.

SUMMARY.

The study of lesions excised from eleven cases of varicella in adult Filipinos shows that the initial change consists in the appearance of peculiar eosin-staining inclusions within the nuclei and cytoplasm of epithelial and various other cells. Direct division of nuclei without subsequent division of the cytoplasm is associated with these inclusions. Cells undergoing these changes often attain relatively enormous dimensions (the ballooning degeneration of Unna). This type of degeneration is most prominent in the affected areas of the epidermis but occurs also in almost every type of cell in the corium. The epidermis presents also the reticulating type of degeneration, but only in a minor degree.

Following these cell changes the typical varicella process consists of a rapid destruction of small areas of the epidermis, associated with exudation and vesicle formation. The exudate penetrates the injured area of the epidermis, forcing the degenerating cells apart and forming spaces which tend to coalesce to form a single chambered vesicle. Exudative cells probably of endothelial origin are occasionally met with in the epidermis very early in the process. Collections of similar cells are found about the blood vessels of the underlying corium. Later in the process they are present in the corium in large numbers and exhibit

amœboid activity and phagocytosis. The exudate filling the vesicle is at first clear and contains only fibrin and a few degenerating or necrotic epithelial cells, but later large numbers of phagocytic endothelial cells are found in it. The active destructive process is followed at once by repair. The epidermis grows in, closing the defect, and the corium returns to its normal conditions.

From the wider distribution of the process in the corium than in the epidermis and from the occasional occurrence of a process in a blood vessel far removed from any epithelial focus, it seems probable that the first change in the corium antedates that in the epidermis.

The development of the vesicle is attended by the formation, about the epithelial cells, of numerous fibers which agree very closely with the fibers of Herxheimer found in the normal epidermis. It is apparent, however, that there is actually an increased production of these fibers in epidermis stretched through the pressure of the exudate.

Several varieties of atypical lesions occur. The exudate may break into the wall of the vesicle and separate the horny layer so that a secondary vesicle is formed which includes the original, or lesions may occur practically without exudation. Some lesions are confined to the depths of hair follicles without the surface epithelium being affected. A certain number of lesions are infected by bacteria as the result of which they become pustular. In such lesions there is more or less destruction of the papillary layer which accounts for the scars frequently following an attack of varicella.

Specific nuclear and cytoplasmic inclusions are found in all varicella lesions and their appearance constitutes the earliest change observed in the tissues. They occur in epidermal cells and in various cells of the corium. The nuclear inclusions stain red or purplish by the eosin-methylene-blue method, and vary in size from 1 to 6 microns in diameter. The cytoplasmic inclusions stain a deep purple and a central granule is apparent in many. They are seldom found measuring over 4 microns. A conclusion has not been reached concerning the nature of these inclusions in varicella and no important evidence has been found in favor of the hypothesis that they are parasitic organisms.

Inoculations of the cornea of the rabbit and the skin of the monkey with the contents of varicella vesicles have in all cases yielded negative results.

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ILLUSTRATIONS.

PLATE I.

FIG. 1. A minute early lesion. The epidermis is thickened over a small area, the result of the swelling of cells. The nuclei are large and vesicular and several contain the characteristic inclusions.

2. An early varicella lesion showing multiple centers of degeneration in the epidermis. The ballooning type of degeneration is most prominent. Exudate has penetrated the epidermis, separating the cells and forming small chambers.

3. A minute varicella vesicle presenting a defect in the epidermis forming its floor. The exudate contains a small amount of fibrin. Considerable infiltration of the underlying corium.

4. The process affecting the epidermis about the duct of a coil gland, which, however, is not involved. Three large multinuclear cells resulting from direct nuclear division are present in the degenerated portion of the epidermis.

5. A minute lesion taken within twenty-four hours after the first appearance of the eruption. Exudate has penetrated the degenerated epidermis forming small spaces. This represents the beginning of vesicle formation.

6. A typical unilocular vesicle taken twenty-four hours after the first appearance of the eruption. The fluid filling the chamber contains a small amount of fibrin and a few epithelial cells which have became loosened in the exudation. The epithelium persisting beneath this chamber presents various degrees of ballooning degeneration.

PLATE II.

FIG. 7. Vesicle taken twenty-four hours after the first appearance of the eruption with lense shaped chamber extending, through the axis of which is a hair sheath. The fluid contents of this vesicle appeared perfectly clear macroscopically, but contains a small amount of fibrin. Slight infiltration about the vessels of the corium.

8. A lesion located in the depths of a hair follicle and presenting several large multinuclear epithelial cells. The surface epithelium is not affected.

9. Two characteristic cytoplasmic inclusions situated in the epidermis. X 1000.

10. Coarse fibers found in the epithelium forming the wall of the vesicle. X 1000. Stained with phosphotungstic acid-hematoxylin.

11. Flaccid, slightly clouded vesicle of two days' duration. Large amount of fibrin in exudate. Epidermis involved in lesions almost completely necrotic.

12. A large nuclear inclusion found in a sebaceous gland. Some indication of internal structure. X 1000.

13. Vesicle of four days' duration. Fluid contains large numbers of leucocytes and phagocytic cells. Epidermis is commencing to grow in at the sides.

14. Corium in the vicinity of a varicella vesicle. The pigment cell to the left of the capillary contains a large number of nuclei probably resulting from (a) mitotic division.

PLATE III.

FIG. 15. A vesicle of six days duration. A break has occurred in the lateral wall of the vesicle at some time during its development allowing the fluid to penetrate and separate the horny layers. A coil gland duct extends down from the floor of the vesicle. Repair is well advanced and the epithelium is growing over the defect upon which the vesicle is situated.

16. A minute "dry" lesion showing advanced degeneration of the epithelial cells without any appreciable fluid exudate.

17. Multinuclear epithelial cells shown at lower magnification in Fig. 4. Outline irregular, without definite cell membrane; cytoplasm presents a loose reticulum; nuclei hollow, with chromatin situated near nuclear membrane, and usually containing the specific inclusions.

18. Cells, probably endothelial in origin, situated in corium. They show localized swelling of the nucleus about the specific inclusions.

19. An atypical vesicle, multilocular in type and showing a central crust. The base is infiltrated with leucocytes.

PLATE IV.

[Camera lucida drawings with Zeiss compensation ocular No. 6; objective, 2 millimeters; aperture, 1.30.]

FIG. 20. Tissue about a small blood vessel of the corium just beneath an early varicella vesicle. An endothelial cell in situ upon the vessel wall contains a small inclusion within its nucleus. Other nuclei in the tissue about the vessel contain the characteristic red staining inclusion situated in clear spaces or vacuoles in the nucleus.

21. Skin excised early in the disease. The epidermis of the Filipino contains a large amount of pigment. A single endothelial cell containing a nuclear inclusion is shown. Another endothelial cell found in another optical plane and also containing a nuclear inclusion is not shown. The epithelial cell above shows a characteristic nuclear inclusion. The study of serial sections failed to show any other similarly affected cells in this portion of the epidermis so that it is to be regarded as a very minute lesion.

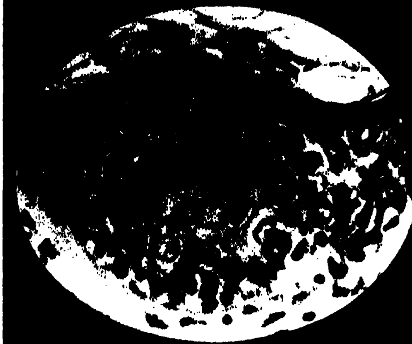
22. Nuclear and cytoplasmic inclusions in the cells of both corium and epidermis.

23. An epithelial cell possessing two nuclei, one of which appears hollow and contains a red stained body. Just outside the nuclear membrane is a more intensely stained body.

24. Epidermal cells in the floor of developing vesicle. Amitic nuclear division has here resulted in a large multinuclear cell containing inclusions in several of its nuclei, and also one extra nuclear inclusion. Other inclusions are present in adjacent cells. Some of these inclusions, especially the cytoplasmic, have deeply stained central points.

25. Cells from a sebaceous gland through which the knife passed in excision. Many of the nuclei containing inclusions have been ruptured. The inclusions are relatively large and show in certain instances a suggestion of reticular or vacuolar structure. The deeply stained granules present in two may possibly be superimposed or included chromatin from the cell nucleus.

26. An epidermal cell from a varicella vesicle showing the extension of a portion of the specific nuclear inclusion through the nuclear membrane.



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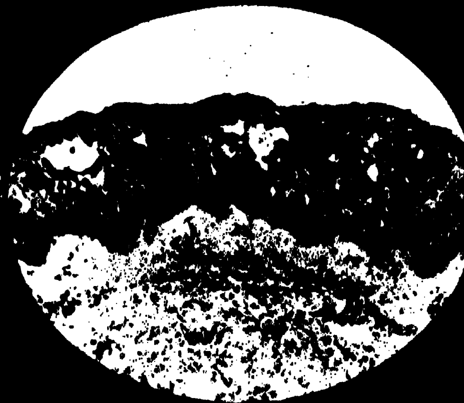

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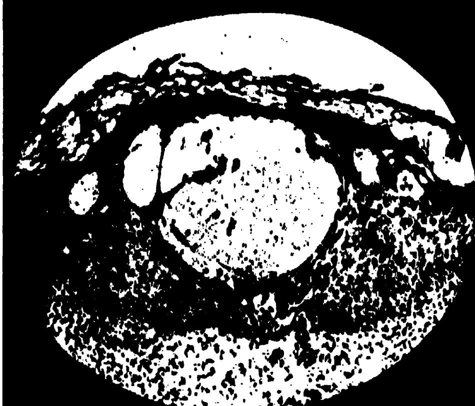

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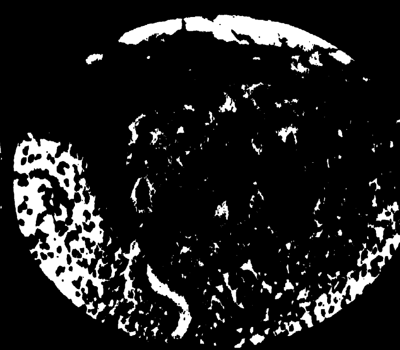

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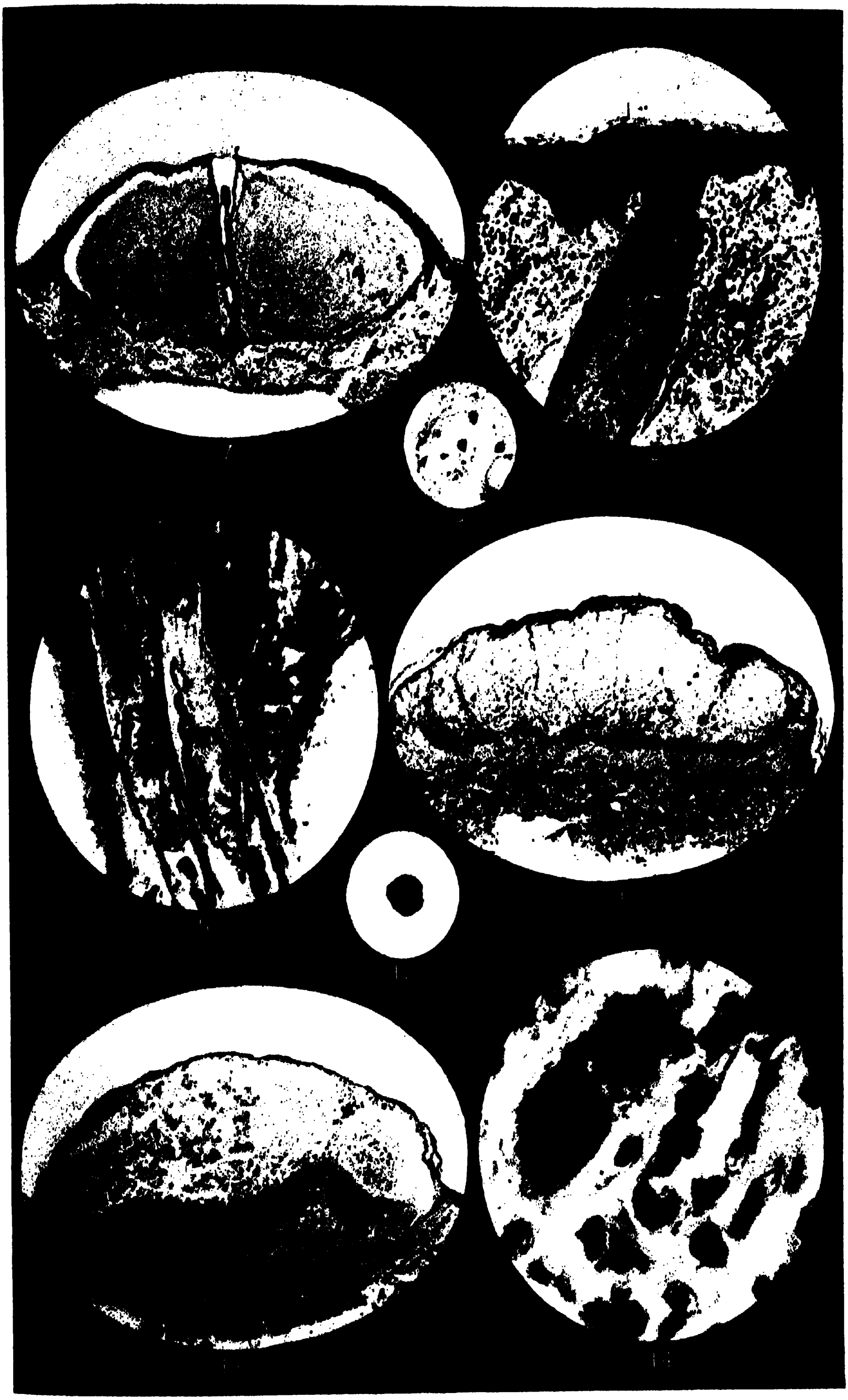


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THE VEGETATION OF THE LAMAO FOREST RESERVE.

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INTRODUCTION.

It is only within the last decade that the science of ecological plant geography has come to be recognized as distinct. It is therefore pertinent to review briefly the history of its development. Attention will be called only to the principal contributions.[1] Floristic plant geography will not be considered.

Grisebach [2] was the first to point out that the unit of vegetation is the *formation*. This he defines as a group of plants which bear a definite physiognomy, as a forest, a desert, or a prairie. Thurman [3] emphasized the necessity of ground water as a basis for classification and Warming [4] first clearly showed the connection between the former and formation, whereas Schimper's [5] contribution lay in a discussion of the influence of the availability of the ground water. Thus salty and cold soils were classed with dry ones because their effect upon vegetation is the same; viz, all tend to produce xerophytic structures. Up to this time the idea of *succession*, although it had been recognized as an element, had not been taken into consideration as a basis for classification. Cowles [6] was the first clearly to show that the dynamic element of vegetation brought about a succession of formations and his classification is based on the idea that habitat changes with resulting modification in the conditions for plant growth and hence an alteration in plant formations to conform with the new physical factors. Adapting to plant formations the modern

[1] For an ecological bibliography see Clements, E. C. Research Methods in Ecology. Univ. of Nebraska Pub. Co. (1905).

[2] Grisebach, A. Bericht über die Leistungen in der Pflanzengeographie (1843).

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idea that each topographic type has a life history, an origin, youth, middle age, and death, he first conceived of the formation as an organic whole, which is susceptible to any change modifying its environment. He laid emphasis upon the fact that the normal erosive forces change the relation of the ground-water level and so, as base level is approached, continually bring it nearer to the surface, by which means water becomes more and more available for plants. By all these workers ground water is recognized as a controlling if not the primary factor. None of the authors cited have made any serious attempt to measure the water content of the soil and the other physical factors controlling plant distribution. It remained for Clements[7] to perfect suitable instruments with which to measure the factors and, as a result of his investigations, the concept was formed "that vegetation is to be regarded as a complex organism with structures and with functions susceptible of exact methods of study." This author also developed a system of nomenclature which, with possible changes, bids fair to find acceptance by ecologists. His classification is based primarily on the physiological ground-water which he calls the *chresard*, by which form he designates the actual water in the soil which is used by the plant. This is measured in the field and because of a limit to the size of the root mass and its surrounding earth which can readily be handled, only herbaceous or small woody plants can be utilized. How the chresard of larger plants, too bulky to be handled, is to be estimated the author does not state and unless some method is provided whereby the chresard of the larger, woody plants can be measured *in situ*, or at least computed, the true chresard of forest formation can not be obtained.

It is obvious that the chresard varies from season to season. In temperate zones it is high during the rainy months of the growing season, lower during the dry ones, and least during those of winter. If, as Clements has done, the average of all the summer months be taken, and a classification based on this alone, then the winter condition will be left out of consideration and any classification which does not take into consideration the lowest chresard can not find a general application. There must be a recognition of the tropophytic nature of vegetation and of an unfavorable and favorable season. This is the case not only in temperate but also in tropical regions. In regions of the Tropics where the physical conditions, especially the rainfall, are more or less uniform throughout the year, the factors measured during one month will give an average for the year, but some places in the Tropics have pronounced wet and dry seasons. If the chresard measurements should

[7] Clements, l. c. I have not access to Clements's Development and Structure of Vegetation. *Bot. Surv. Nebr.* 7 (1904). His volume (in preparation) on the vegetation of the mountains of Colorado may throw some light on the discussion which is given below. I have obtained my information concerning his methods from the volume quoted at the beginning of this paper.

be taken during the wet season only (corresponding to the summer season of temperate regions) they would be grossly misleading. It might be assumed that the winter chresard of temperate regions is nothing, but the physiological evidence is entirely against such an assumption. Coniferous trees are known to use water throughout the so-called unfavorable season and even the bare twigs of deciduous trees may give off water. In many portions of the temperate regions the conditions are such that vegetative activity is considerable during the winter and especially the very early spring months. Granted that the chresard is the controlling factor, *no general classification based upon exact methods of measurement can be made until the measurements show chresard conditions during the unfavorable as well as the favorable season.*

However, Clements's work marks a new epoch in ecological studies. In a word, it reduces ecology more nearly to a physiological basis and some of the mooted questions raised in this paper could have been decided had his methods been adopted. Nevertheless, it is my belief that much preliminary work can be done and indeed is necessary before careful measurements are made. Especially is this true in a region where little is known of the floristic composition of the vegetation. Attention must be focused on obtaining a knowledge of the flora, including not only an enumeration of the species but also a determination of the quantitative importance of each. Without this, no progress can be made toward an explanation of the ecological factors controlling distribution. Although the vegetation of the strand in the Tropics has been fairly well studied, but little is known of the composition of inland formations in these regions. The quantitative analysis of some forty plots, the results of which are given in this paper in such a way as to show what vegetation exists in different physiographic situations at different altitudes, is therefore a distinct contribution. During this study an effort has been made to discover what factors control distribution. How successfully this attempt has been realized will be shown by the paper which follows.

It is not too much to say that ecological botany, more than any other branch of the science, has a practical as well as a scientific value. Stripped of its business aspects, forestry is nothing more or less than forest ecology. One of the first essentials of forestry is a knowledge of the composition of the forest and the only scientific means of obtaining this knowledge is by a study of plots similar to those recorded in this paper. Indeed, with the addition of tree measurements, these plots are the "valuation surveys" of the forester. In many places in the Philippines, especially along the coasts where transportation is easily available, local forest famines already prevail. The valuable timber trees have been removed and "weed" trees have taken their place, or clearings have been made, found to be unprofitable, and have been abandoned and a knowledge of the natural succession of vegetation in these places is an

absolute essential to any rational treatment which will again bring them into a good forest condition. The above discussion emphasizes only a few of the many questions which the forester must answer concerning the ecology of the forest before his calling is reduced to a scientific basis.

The foundation work of crop raising, like that of forestry, is ecology. The *habitat* of the ecologist is the *field* of the farmer. The more the farmer knows of the factors of this *habitat* the better will he be able to reduce his farming to a science, and until he makes an attempt to discover something concerning this habitat, his farming will be guess work. Unsystematized chemical and physical analyses of the soil, without any relation to the plants grown thereon, are of little or no value. The *formation* of the ecologist is the *crop* of the farmer. The factors which control the one will govern the other with this difference, that, whereas under natural conditions the waste in fallen leaves, twigs, etc., is each year returned to the soil in its entirety, in artificial vegetation the greater part is consumed, so that eventually, unless some return is made, the soil becomes depleted.

The advisability of differentiating forest from agricultural lands can not be questioned in any partially undeveloped country like the Philippines. Ecological surveys will not only do this but if they are made with due regard to the careful measurement of the physical factors, they will give a clear classification, the results of which when placed on maps will show at a glance the economic possibilities of the region investigated.

The systematic nomenclature of this paper is the one used by E. D. Merrill in his "Flora of the Lamao Forest Reserve,"[a] which is to be published simultaneously with this paper. I am indebted to Mr. Merrill for most of the identifications. Besides the various members of the Bureau of Science who have assisted me in the preparation of this paper, I am also indebted to Capt. George P. Ahern, Chief of the Bureau of Forestry, and a number of employees of his Bureau for valuable aid rendered while I was collecting the data. Where special assistance has been rendered, acknowledgment will be given in its proper place. The map accompanying this report was adapted from certain ones of the Coast and Geodetic Survey, and from a topographic map of the reserve made by the Bureau of Forestry.

GEOLOGY AND PHYSIOGRAPHY.

The group of peaks known as Mount Mariveles is on the southern end of the peninsula of land comprising the Province of Bataan, of the Island of Luzon. The meridian of 120°.30′ east of Greenwich and the parallel of 14° 30′ north latitude intersect the mountain near its summit. Rising to the height of approximately 1,400 meters, it, with the Island of Corregidor, is the most conspicuous feature of the landscape at the entrance to Manila Bay. (See map.)

[a] *Phil. Journ. Science* (1906) 1, Supp. 1, April 15.

The lithologic structure of Mount Mariveles shows it to be of volcanic origin. It is a mass composed largely of andesitic ejecta and of the decomposition products thereof.[9] The physiographic features which point to its being an extinct volcano are almost as striking. Rising from Manila Bay on the east, from the China Sea on the south and west, and from the lowlands near the central part of Bataan on the north, are a series of prominent ridges ending in the peaks which, with their connecting ridges form a nearly circular chain—the rim of the former crater which has an outlet to the north, by way of the Balanga River. (See map.) Between these peaks and just beneath the lowest point of the connecting ridges are the sources of the main rivers draining the mountain; the latter have cut deep cañons and have established many branches with smaller ones. With the exception of Limay Peak, which will be discussed in another connection, the mountain, with its regular cañons and ridges which begin at the base and end at the rim of the crater, suggests that in the past it was a more or less perfect cone, considerably higher than at present and similar to Mount Mayon, in the Province of Albay, Luzon. According to Becker,[10] the volcanic period which gave rise to the earlier andesitic rocks now present in the Philippines very probably occurred in the Post-Eocene upheaval. If Mount Mariveles originated at this time, then the present erosive topography of the mountain is no older than the length of the interval intervening between the Miocene and the present date; probably it is considerably younger, for the volcano must have remained in activity for some time after its origin. At present there are no signs of volcanic activity if we except some hot springs near the base of the mountain at Port Mariveles.

The condition of the mountain to-day is only one stage in the process of its destruction by the normal erosive forces. It is conceivable that its vegetative condition, because of its prominent topography of alternating ridges and cañons, is considerably different from that of some other volcanic peaks on the Islands which are in various stages of their life history. The youthful Mayon, with its cone-like, little eroded topography, or Mount Banajao, in Tayabas Province, with a physiographic condition intermediate between that of Mayon and Mariveles, will show vegetative conditions due to factors to some extent at least dependent on their respective life histories.

Lamao River Reserve.—The portion of Mount Mariveles with which this paper deals lies on its east slope and comprises an area of approximately 4,426 hectares. It is known as the Lamao River Reserve, and has

[9] I am indebted to H. D. McCaskey, of this Bureau, for information concerning the lithologic structure of the mountain. See also Becker, Geo. F. Report on the Geology of the Philippine Islands. *Annual Report of the U. S. Geog. Sur.* (1901) 21, III, 514.

[10] *L. c.*, 567, 568.

a water frontage on Manila Bay of nearly 5 kilometers, extending from Cape Magarhas on the south, in a northerly direction, to Cape Quitang. These two points mark the lower ends of the main ridges which respectively are the northern and southern boundaries of the Reserve.

The northern ridge extends from Cape Quiling westward on the ridge leading to Buenavista Peak (*altitude*, 1,165 meters); from thence it follows the rim of the crater to Cabcaben Peak (*altitude*, 1,406 meters). (Pl. I.) The southern boundary is the ridge which starts at Cape Margahas and runs through Limay Peak to Caybubu Peak (Pl. II) (*altitude*, 1,386 meters) and on the rim of the crater. The western and northwestern boundaries of the reserve are formed by lines which descend from Cabcaben on the south and Caybubu on the north, meeting at the bottom of the crater near the head waters of the Balanga River.

Between the north and south ridges are two main rivers—the Lamao and the Alangan. The Lamao River rises just beneath the lowest point of the rim of the crater which connects Buenavista (Pl. II) with Caybubu Peak. From its source down to within 3.5 kilometers of the shore, it lies in a cañon which varies in depth from 75 to 270 meters; below 3.5 kilometers the latter feature of the river disappears. Throughout its length it is an eroding stream, with alternating cascades and pools; near its source waterfalls of 15–20 meters and perpendicular walls 30 to 40 meters in height are prominent features; the breadth of the river at no place exceeds 12 meters. Four main branches, a number of wet-weather streams, and numerous ravines cut the slopes of the cañon of the main stream into many steep and shorter ridges and cañons; some of the details of this topography are shown by Plate II. The cañon feature of the Alangan, although not so pronounced as that of the Lamao River, is still marked. It has not been successful in working its way to the rim of the crater; indeed, the Camayuan River, a branch of the Lamao, has its head waters above that of the Alangan and collects drainage which would otherwise be diverted to it. Thus the drainage territory has been divided between two rivers and this fact has prevented either one from obtaining a sufficient supply of water so as to make cañons as deep as would one river, draining the same territory, and as a consequence the topography is not as deeply dissected as it otherwise would be. The cone-like contour of Mount Mariveles is slightly disturbed by Limay Peak, the altitude of which is 978 meters. It may either be a more resistant portion of the ridge leading from Cape Margahas to Caybubu Peak, or a portion of a rim of a smaller crater. The region to the north of it was not investigated with sufficient care to determine its true nature.

Between the Lamao and Alangan Rivers the lower third of the reserve is drained by branches of both, by the short Ayam River, and by many ravines and wet-weather streams which have their outlets in the indentations of the shore line. These dissect the gently sloping, low plateau at the base of the mountain into a number of low ridges and shallow depressions.

THE CLIMATE AND ITS INFLUENCE ON THE VEGETATION.

The classification of the vegetation used in this paper is based upon its character, and the climatic differences of the region. The climatic records for Lamao given below were gathered during a portion of my residence at that place: of themselves they are of little or no value, but comparatively, their usefulness in explaining some of the more marked

characteristics of the vegetation is great.[11] They will be considered in the order of their relative importance.

Humidity.—Schimper[12] bases his climatic formations mainly on rainfall; those of the tropical lowlands fall under two heads—the tropical rainy and the tropical monsoon forests. The former are found in places where evergreen forests prevail during almost the entire year; the latter are those in which the dry districts are occupied by deciduous woodlands or savannahs. From the above it will be seen that the tropical rain forests are not necessarily those having precipitation which is uniform throughout the year; any alternation of wet and dry periods will show corresponding marked differences in the vegetation.

TABLE I.—*Average monthly rainfall, in millimeters, at Manila, 1865-1902.*

Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Total.
58	29	9.8	18.6	28.4	101.6	247.6	381	361.2	374.7	191.8	136.5	1,938.3

TABLE II.—*Rainfall, in millimeters, at three stations at Lamao compared with that for the same period at Manila for a part of the year 1905.*

	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.
Station 1	t		0	0.8	127	38.1	197.2	1,071.8	270.5	425.4
Station 2			t	16	139.7	40.4				
Station 3	....		2.2	6.8	273.3	70.1	1,034.3	1,401.6	291.6	709.2
Manila	20.2	0	2.8	1.1	[13]173.8	24	346.2	591.4	212.8	239.6

In this and in the following tables station 1 is at sea level, station 2 at an elevation of 85 meters, 4 kilometers inland, and station 3 at an elevation of 640 meters, 13 kilometers inland. These two tables conclusively show well-marked wet and dry seasons both for Manila and the stations at Lamao. So slight is the rainfall during the dry season that one is surprised to find a forest in which the evergreen is as characteristic as the deciduous element and not a completely deciduous one to correspond with this climatic condition. During the season of little or no

[11] The necessity of a base for comparative climatic data can not be too strongly emphasized. Isolated readings without such a comparison are practically worthless; on the other hand, with such data their value becomes nearly as great as would be that of continuous records. However, the readings must be made simultaneously. For the Manila records up to 1903 given here, see Algué, Rev. Jose. Climate. *Census of Philippine Islands*, 1903, 1: 87. I am indebted to R. Meyer, T. Hanley, and other employees of the Bureau of Forestry for assistance in the collection of climatic data.

[12] *L. c.*, 160, 261.

[13] The excessive rainfall for April is the highest ever recorded. It is mainly the amount which fell during the typhoon which visited the Islands on the 30th of that month.

rain the surface layers of the soil are dry and the ground water level is low, yet, it is believed, a sufficiently high chresard exists to prevent the most pronounced deciduous habit from prevailing and vegetative activity from being altogether inhibited. The table shows that with an increase in altitude there is a decided increase in the rainfall. This, among other things, will account for a chresard which would be sufficiently high to assist in maintaining a nearly evergreen forest on the upper part of the mountain, although here, as well as at the base, there is a response to the xerophytic conditions of the dry season. On account of its deciduous element, the vegetation at the foot of the mountain can not be regarded as the best expression of Schimper's tropical rain forest, neither can it be classed with the monsoon forests, for the evergreen element is nearly absent in the latter.

Next to the rainfall, and especially to the precipitation as conserved in the shape of a chresard, the relative humidity (or its complement, the saturation deficit) is the most important factor in explaining ecological distribution, for just as the chresard controls the absorption, so does the saturation deficit regulate the transpiration.

TABLE III.—*Monthly and annual averages of the saturation deficit for Manila, 1883–1902.*

Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
19.3	22.4	25.8	28.2	29.8	23.3	18.6	15.2	15.2	14.5	17.3	18

As would be expected, these data show a high saturation deficit during the dry and a low one during the wet season. When compared with those for some regions in temperate zones, these figures are not exceptionally low even during the rainy season. Attempts have been made by Haberlandt,[14] Holtermann, and others to compare the transpiration in the damp Tropics with that in temperate zones. The general conclusion seems to be that in the shade the transpiration in the Tropics is much less than it is under the same condition in the temperate zone, while in open places it is greater. Holtermann draws the conclusion that for the entire twenty-four hours of an average day it is less in the Tropics than in Europe. In the region under consideration, especially during the month of July, there is almost continuous rain for two and even three weeks, yet these rains are usually accompanied by high winds and the periods between the showers show a comparatively high saturation deficit. Considering all factors, it is not believed that the lack of transpiration has any deleterious effect on the vegetation as a

[14] See Burgerstein A. Die Transpiration der Pflanzen (1904) 160 to 174, for a review of the subject of the condition of transpiration in damp warm tropical regions, and for a citation of the literature.

whole, for, up to a certain point, the more water there is retained by the plant the greater the vegetative activity.

TABLE IV.—*Average saturation deficit for Lamao and Manila at 1 p. m.*

	Feb.	Mar.	Apr.	May.
Station I	39.7			
Station II	38.1	30.8		31.3
Station III	30.8	28.6	31.7	13.4
Manila	40.7	46.7	45.8	41.8

A comparison of the saturation deficit at the foot of the mountain with that of Station III shows an increase as one ascends. These data were taken at the time of the year and of the day when the transpiration is excessive. It is believed that the comparatively low saturation deficit together with the lower temperature, the effects of which will be discussed below, and the greater rainfall, help to maintain the more pronounced evergreen character of the vegetation on the slopes of the mountain in contrast with that at the base. A record of the maxima during some months of the dry season may not be out of place and it will throw some light on the differences at different altitudes. On February 26, at Station I, the saturation deficit was 53 at 2 p. m.; at the same time it was 32 at Stations II and III. The highest recorded in March was on the 1st day of that month, when it was 49 at Station II and 42 at Station III. In May on the 14th it was 42 at Station II and 16 at Station III.

Temperature.—The following tables give the comparative temperature of Manila and the various stations at Lamao (readings are in centigrade):

TABLE V.—*Mean monthly and annual temperature for Manila, 1883–1902.*

Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Average.
25	25.4	26.8	28.3	28.6	27.9	27.1	27.1	27	26.9	26.1	25.2	26.8

TABLE VI.—*Monthly and annual maxima of temperature in Manila,*[15] *1883–1902.*

Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Average.
32.1	32.9	31.3	35.5	36.1	35	33.5	33	33.3	33.3	32.2	32.1	36.3

TABLE VII.—*Monthly and annual minima of temperature in Manila,*[16] *1883–1902.*

Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Average.
17.9	18.0	18.5	20.8	22.7	22.6	22.4	22.3	22.4	21.4	20.3	18.3	17.1

[15] The *highest* recorded temperature for Manila is 37°.8.
[16] The *lowest* recorded temperature for Manila is 15°.7.

TABLE VIII.—*Average monthly temperature of three readings (7 a. m., 1 p. m., and 6 p. m.) in Manila and Lamao for a portion of 1905.*

	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.
Station I	25.7	28.5	28.5	29.7	29.6	26.6	26.5	
Station II	25.8	25.2		28.5				
Station III	21.0	22.6	24.5	23.4	22.7	21.9	23.1	22.8
Manila	25.4	28.0	29.5	29.6	28.	22.7	27.6	27.0

TABLE IX.—*Absolute monthly maxima of temperature (1 p. m.) in Manila and Lamao for a portion of 1905.*

	Feb.[17]	Mar.	Apr.	May.	June.	July.	Aug.	Sept.
Station I	32.4	35.8	36.0	36.5	34.0	34.0	32.5	
Station II	30.5	34.0		32.6				
Station III	24.6	27.0	28.6	28.0	26.0	26.2	26.2	26.0
Manila	32.5	34.9	31.7	35.5	34.4	31.9	33.0	33.9

TABLE X.—*Monthly minima of temperature (7 a. m., 6 a. m., or 6 p. m.) in Manila and Lamao for a portion of 1905.*

	Feb.[17]	Mar.	Apr.	May.	June.	July.	Aug.	Sept.
Station I	20.0	20.8	25.0	24.0	24.0	23.0	23.0	
Station II	18.0	19.0	22.5	23.8				
Station III	17.8	18.5	20.0	22.0	20.2	20.0	21.0	20.2
Manila	18.0	20.2	23.7	24.8	24.0	22.7	23.8	24.0

These data in regard to temperature show, within each station, a rather uniform monthly average, the extremes being farther apart in the lowlands than they are on the slope of the mountain. In all cases the minima are not low, although at the higher altitude the average is much lower than near sea level. From an ecological standpoint this is important. At the same time it must be remembered that there are no physiological experiments which show that heat directly influences structure in plants; in other words, there are no ecological adaptations in them which can directly be traced to heat as their cause. Plants found in all conditions of temperature often have similar habits, all of which are due to their water and not to their heat relations.

Indirectly, temperature has a great ecological effect by influencing the condition of the atmospheric or of edaphic moisture, which control the ingress and egress of water into and from the plant. Thus, a diminution of the temperature below a certain limit lowers the chresard. However, the temperature of the region under consideration is not sufficiently low materially to influence the latter, for in the Tropics it is only in the

[17] After February 17.

very high mountain regions that low temperature is likely to bring the chresard below the danger point. The saturation deficit is the direct measurement of the influence of the temperature on the condition of the atmospheric moisture, so it is not necessary again to consider it in this connection.

However, independently of the chresard and of the atmospheric humidity, heat influences the transpiration and thus may effect ecological structure. The chresard and saturation deficit remaining constant, a raising or lowering of the temperature respectively will increase or decrease the transpiration. A reference to the tables will show a continually lower temperature on the slopes of the mountain as compared with that at the base.[18] It is believed that this element, together with the lower saturation deficit and the greater rainfall, must be taken into consideration to explain the almost evergreen character of the forests on the mountain slopes, as compared with their decidedly deciduous aspect at the base of the mountain.

Aside from its ecological aspect, temperature has a profound influence on the floristic character of the vegetation, so that the upper regions of the mountain under consideration have floristic elements different from those of the region near the level of the sea. This contrast will clearly be shown when the plant formations are considered in detail.

Wind.—Wind is another factor affecting ecological distribution. In the absence of proper instruments no record of wind velocity was kept at Lamao. The effect of wind on vegetation, especially in exposed situations, will be discussed in another connection.

TABLE XI.—*Monthly and annual averages of the daily velocity of wind in Manila during the period 1885–1898, in kilometers.*

Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
165.9	192.8	224.8	232.7	231.4	234.5	280.0	273.8	296.0	182.3	151.5	149.3

Average, 217.8.

SOIL.

The soil of the Lamao Reserve is remarkably uniform in its physical texture and chemical composition. With the exception of that on the beach, it is all of a residual nature from the andesitic rocks which form the core of Mount Mariveles. It is usually a heavy clay, more or less impregnated with iron, which resembles laterite. However, it is not at all the laterite of Burma,[19] which is remarkable for its deficiency

[18] For literature on the effect of air temperature on transpiration see Burgerstein, l. c., 115 to 121, *especially Eberdt, O.* Die Transpiration der Pflanzen und ihre Abhängigkeit von äusseren Bedingungen (1889).

[19] Schimper, *l. c.*, 379.

in certain salts, and, what is more important ecologically, its incapacity to hold water, and which supports the famous "eng" (*Dipterocarpus tuberculatus*) forests in that region. The soils of Mount Mariveles are not deficient in essential mineral salts and they show a very high capacity for retaining water. Tests made of representative specimens of the soil show that its average capacity to hold moisture is 53 per cent in weight of water, calculated on the total volume of air-dry soil. It is very probable that this power has a great deal to do with its capability of maintaining a sufficiently high chresard during the dry season, during which time the underground water level is lowered and the supply of water brought by capillarity to the surface layers is consequently less.

There is no serious contention that the chemical constitutents of soil has any pronounced effect on ecological distribution, for the reason that nearly all soils contain the essential elements in sufficient quantities.[20] Exception must be made to the deleterious nature of strongly alkaline and salty soils, and even here the effect is physical rather than chemical. In undisturbed conditions, with the exception of the amount temporarily stored up in new growth, practically all which is taken from the soil by plants as mineral salts, is returned thereto in the shape of fallen leaves, twigs, etc.; it is only in cultivated ones where the crop is removed that they become worn out.[21] Attention will be called to peculiarities of the soil under the heading of formations.

THE PLANT FORMATIONS.

The types of vegetation on the Lamao Reserve may be divided into six formations; viz, the *Strand*, the *Bambusa-Parkia*, the *Anisoptera-Strombosia*, the *Dipterocarpus-Shorea*, the *Shorea-Plectronia*, and the *Eugenia-Vaccinium*. (See map.)

I. THE BAMBUSA-PARKIA FORMATION.

Character of the formation.—The vegetation at the base of the mountain has been given the name *Bambusa-Parkia* because it is believed that in the genera *Bambusa* and *Parkia* are exhibited forms of plants which best indicate the response in the vegetation to the ecological conditions which are obtained in the habitat in which these genera prevail. The response is such as to meet the almost xerophytic conditions present during the dry season. The genus *Bambusa*, represented by several species, has some of the ecological advantages of trees and many of those

[20] For a review of the literature on this subject see Cowles, H. C. The Influence of Underlying Rocks on the Character of the Vegetation. *Bull. Am. Bureau Geography* (1901).

[21] See Livingston, B. E., Britton, J. C., and Reid, F. R. Studies on the Properties of an Unproductive Soil. *U. S. Dept. Ag., Bureau of Soils Bull.* (1905) 28. In this paper it is maintained that "worn-out" soils may be poisoned ones.

of grasses. Often reaching to a height of 15 or 20 meters, the bamboo is able successfully to compete with the dicotyledinous trees of the same height. This advantage is best utilized during the favorable season, when conditions for growth and photosynthesis are at their maximum. While none of the bamboos of the Lamao Reserve are completely deciduous during the dry season, yet the total area of the foliage is much less then than during the wet one. This is brought about in two ways. Many of the leaves of individual shoots are shed, leaving them with from one-third to one-half as many leaves as they would have during the rainy season. In this way the bamboos simulate the semi-deciduous trees with which they grow. Those which remain may reduce their transpiration surface by curling up like grass. Again, some of the *culms* die down altogether during the dry season, thus reducing the transpiration surface of the clumps from which they spring. Grasses may either form sod by sending out runners or underground shoots which radiate in all directions from a common center, or they may be caspitose, when they are commonly known as "bunch" grass. Bamboos, like other grasses, may be represented by species of both types, although the "bunch" type is the predominant one on the Lamao Reserve. While the individual shoots are short-lived, the permanency of the "bunch" is maintained. To sum up, the half-grass and half-tree like habit of the bamboo is peculiarly fitted to a formation which is midway between the best expression of Schimper's tropical rainy and his tropical savannah forest.

Parkia roxburghii, the tree after which the dicotyledinous portion of the formation is named, is only one of a number which is deciduous a part of the dry season. (Pl. III.) While not so abundant as some of the other species of the same type, yet it is the largest and most conspicuous one present.

Within the *Bambusa-Parkia* formation there are many places where the bamboo element is altogether absent. This is more true of *Parkia roxburghii,* although the *Parkia* type (deciduous) is nearly always represented by some species. Careful measurements of the chresard will no doubt show constant variations in different portions of the formation although, except for a narrow margin along the banks of the rivers, these differences are not thought to be great enough to justify a subdivision of this formation into two or more coördinate ones. Rather, they are all considered to be stages, physiographic or artificial, in the life history of the vegetation which the region is able to support and which is a mixture of the bamboo and of the semi-deciduous types expressed by the names of the formation.

The map shows the general limits of the formation. Briefly, it extends from the upper limits of the littoral vegetation to a varying height on the side of the mountain. Making a liberal allowance for a tension line (ecotone) between this and the adjoining formation, it reaches an altitude of 75 meters on the south side of the reserve, while it runs up

the slopes of Limay Peak as high as 175 meters. Toward its limits on the south side, tongues of it extend farther up the lower slopes of the ridges, while on the north side it seems to occupy both the tops and the slopes thereof. Here it is especially well marked on the more exposed ends of ridges having abrupt termini. The terrace of the Lamao River may or may not have isolated patches, but usually it is absent in these places. The region near the sea shows only isolated clumps of the indigenous species of bamboo, although here the dicotyledinous trees are quite as characteristic as they are farther inland.

The best expression of the *Bambusa-Parkia* formation is peculiar. The species called "boho," probably *Bambusa lumanpao*,[22] is the characteristic form. Clumps composed of fifteen or twenty culms, some dead and some alive, and 12 to 18 meters in height are on an average set at a distance of from 3 to 4 meters apart, not infrequently growing so close together that it would be found difficult to wedge one's way between the individual colonies. These make a shade so dense as practically to prevent the development of herbaceous and woody forms which are not adapted to such conditions. Among the clumps here and there are seen the trunks of isolated dicotyledinous trees. In some places there are groups of three or four individuals, usually of different species, as closely set as the bamboo colonies but more often they are 15 to 20 meters apart. Indeed, if the bamboo growth should entirely be removed and replaced by a grass such as *Saccharum spontaneum*, the aspect of the forest would very much resemble the park-like forests of the savannah or prairie regions. One has only to imagine the grass in the open spaces between the trees in these latter places to be 15 instead of 2 meters high to obtain an approximately correct idea of the aspect which the bamboo forests of the Lamao region presents. As a rule, the dicotyledinous trees overtop the bamboo growth by 6 to 10 and even by 15 meters. There are perhaps as many scattered younger trees as there are older ones which do not reach to the level of the bamboo roof. Independently of the bamboo, the canopy presented by the dicotyledinous tree element shows an extremely irregular profile. The convex portion of the latter is due to the higher semi-circular crowns of the trees overtopping the bamboo; those of the single trees form the smaller convex areas, those of the groups, the larger ones, and these may in turn form smaller irregularities which are due to the differing height of the individuals composing the groups. The bottom of the depressions between the isolated trees or groups of trees is the top of the bamboo colony. During the dry season, especially, the foliage of the bamboo growth shows a browner color than does that of the evergreen element. On the one hand, there

[22] With the exception of the climbing forms, none of the bamboos have been found in flower or fruit. These identifications have been made from vegetative characters.

are the usually gray tops of the deciduous and the dark green of the evergreen trees, and, on the other, the brown tone which accentuates the irregularity of the profile, and so, when it is viewed from a distance at an angle, the park-like nature of the formation is strikingly shown.

The floristic composition varies. Where it borders on the next one above, it partakes of the nature of the latter and imperceptibly grades into it. In a plot 2 kilometers inland, at an altitude of 30 meters, *Albizzia procera* forms a conspicuous element, but, so far as my observations go, this is the only tree which remains deciduous throughout the dry season. Besides *Parkia roxburghii*, the other trees prominent in this plot are *Oroxylum indicum, Zizyphus zonulatus, Bombax ceiba, Lagerstrœmia speciosa, Canarium villosum, Anisoptera vidaliana, Pterocymbium tinctorium, Ficus variegata, Koordersiodendron pinnatum, Wrightia laniti, Adenanthera pavonina, Albizzia saponaria, Premna cumingiana, Mangifera altissima, Aphananthe philippinensis, Artocarpus communis,* and *Buchanania florida.* Among the smaller ones are *Memecylon edule, Streblus asper, Cyclostemon bordenii, Mallotus philippinensis,* and others. Some of the ones mentioned above are deciduous during a portion of the dry season, while others are continuously green, but then they have a much lesser area of foliage than is found during the favorable time of the year. Nearer the *Anisoptera-Strombosia* formation in addition to the species just mentioned are *Shorea guiso, Dipterocarpus vernicifluus, Euphoria cinerea, Strombosia philippinensis, Aglaia harmsiana, Evodia glabra,* and *Talauma villariana.* The less frequent trees are *Endospermum peltatum, Dracontomelum cumingianum, Illipe ramiflora, Sterculia oblongata, Myristica philippensis, Gonocaryum tarlacense, Parinarium griffithianum, Palaquium tenuipetiolatum, Macaranga bicolor, M. tanarius, Carallia integerrima, Alstonia scholaris,* and others. All the species in these last two lists are evergreen throughout the dry season with this exception, that some of them may be completely deciduous for a day or two. All of them show less leaf surface during this time, and the deciduous element is nearly as striking as it is in the region nearer the shore.

The type of the *Bambusa-Parkia* formation, which has just been described probably occupies one-third of the area mapped as such. The other two-thirds come under sub-formations which are considered to be stages in development. As already indicated, isolated groups of bamboo are present throughout and these may or may not be accompanied by the dicotyledinous species named above. The *closed* bamboo forest does not approach nearer than 1½ kilometers to the shore, though "boho" and other bamboos are present near the coast line. One of these, *Bambusa blumeana,* probably an exotic form, is the species used in building operations in the Philippines. It is the tallest of the bamboos, and with its pagoda-like growth forms the most conspicuous feature overtopping the low, shrubby vegetation near the seashore. Whenever the "colonies"

are closely set they form impenetrable thickets. Where *Bambusa blumeana* is planted, the colonies occur in rows along the edges of overgrown clearings.

Effect of artificial disturbances on the formation.—There is both historical and vegetational evidence that much of the area under consideration has been cultivated. Clearings called "caingins" by the natives are merely openings made in the forests by cutting and burning the vegetation. There are all degrees of this destruction, and after the existing vegetation is wholly or partially destroyed, the clearing thus made may perfunctorily be cultivated and then abandoned, or it may be more thoroughly tilled and after a number of years deserted. In any event, whatever the state of the removal of the original vegetation, the natural equilibrium has been disturbed by this means and a new but temporary habitat has been created which allows of the invasion of a type of vegetation which differs from that which has preceded it. Either these clearings may grow up to form grass lands, in which event they may become known as "cogonales," or they may be occupied by arboreal species. The nature of the vegetation which first gains an entrance depends on the extent to which the clearing is cultivated, on the nature of the surrounding vegetation, on the kind of habitat, or on disturbances after the clearing has been abandoned. It should be emphasized that the term caingin is applied to the *place* which is cleared for cultivation. It may remain this name as long as its origin is recognized, no matter what sort of vegetation may invade after it has been abandoned.

Vidal [23] first called attention to a peculiar type of tropical vegetation, prevalent near Manila, and called by the natives "parang." (Pls. III and IV.) His description of the origin of parangs is as follows:

> Abuse in the utilization, which until a very recent time was not subject to any restriction whatsoever, has reduced to shoots the masses of forest which formerly covered the lowlands, eradicating almost all good timber trees. In fact, the aspect of these tracts covered with shoots and saplings differs from the one to be observed in analogous localities of the temperate zones. Being so covered with woody vegetation and young trees, to an unpracticed observer they would appear to be regular woods. Such places are called in Tagalog "parang," a name which should find acceptance in the technical phraseology of the Philippines in the same way as has the word "jungle," which has been admitted by the foresters of British India, because new things must have new names. The "parang" might be defined as an extensive area, covered with brushwood and the trees of the invading species, which have taken the place of those existing before the cutting or burning. The study of these places has a practical importance in the great question of the throwing open of public territory for settlement, because usually they are the most salable of the unoccupied Government lands.

When one species becomes dominant in such places the term may be modified by the native name of the principal tree. Thus, on some old

[23] Vidal, D. Sebastian, *Catálogo Metódico de las Plantas Leñosas Silvestres y Cultivadas observadas en la Provincia de Manila* (1880) 9–10.

maps of Bataan Province one finds such combinations as "Parang de Culis" (*Memecylon edule*) or "Parang de Ligaa" (*Zizyphus*) and others. I can do no better than use this word "parang" as a general term for the types of vegetation about to be discussed, no matter what their origin, and to distinguish one type of parang from another by the generic name of the principal or characteristic species. In caingins which have been abandoned for long periods the invading vegetation often simulates that of the parang to such an extent that at times it is difficult to distinguish its origin. However, in the parang proper exotic species are rarely present, and if present, never predominate.

The laws controlling the invasion of plants into new habitats or into old modified ones, have long been recognized and are more or less clearly defined.[24] It must be remembered that the types of vegetation which are now to be discussed are only stages in the return to a climax formation similar to the *Bambusa-Parkia* formation, which has already been described.

Parangs.—The process of reducing the forest may be arrested at any of its stages. Usually, the original growth is not taken away at one time. If the bamboo is sought, its removal leaves large, open places between the dicotyledinous trees, but all the culms are usually not taken, and even if they are, the food supply stored in the cæspitose bases would enable the clumps to recover their normal condition so quickly that the short respite granted the undergrowth does not enable it to gain much headway. However, repeated and frequent cuttings have a tendency to thin out the bamboo growth, for with a reduction of the photosynthetic surface, less nutritive material is stored in the underground parts, and thus shoots may be removed faster than new material can be elaborated to replace the stored-up food. In this way clumps may be entirely reduced, or at least so much thinned that other vegetation will have a better opportunity to compete. The result is that many species which before were poorly developed, at once spring into prominence. Trees, shrubs, and lianas which were present in the original forest then give character to the vegetation. Of course, if any of these are removed with the bamboo, they, like the bamboo, are not *in situ* to compete with the existing vegetation. The character of the new vegetation will partake of that of the old, minus the species removed.

Dinochloa Parang.—One of the lianas found scattered in the original forest of the *Bambusa-Parkia* formation is *Dinochloa diffusa*, itself a bamboo, with a habit of growth which makes it peculiarly fitted to spread into the open places made in the forests. Where supports are present, like other lianas, it presents most of its foliage in the top of the formation. The stem may branch at long intervals. These branches may

[24] See Clements, F. E., Research Methods in Ecology, 265, for a statement of the laws of invasion of plants into clearings; also Whitford, H. N., The Forests of the Flathead Valley, Montana, *Bot Gaz.* (1905) 39: 282–283.

spread horizontally for some distance and then they may become upright if the necessary support is present. Many branches recline on the ground, in which case movements of soil or gathering of vegetative débris may cover these branches, which then may root at the nodes, and thus, in case older parts are destroyed, new centers originate. While *Dinochloa diffusa* was found in fruit during the two summers under observation, yet very few seedlings were noted; consequently its seedling condition remains yet to be investigated. It will be seen from the above that *Dinochloa diffusa* is well fitted to invade clearing habitats from surrounding places by means of its diffuse habit, or, if already present, it may form dense growths more rapidly in case there is any disturbance in its own habitat. If its tall support is removed, its reclining portions lie prone on the ground or near the ground, if the underbrush is able to support it. In the case where the shrubby vegetation is sufficiently strong to hold the reclining portions above ground, a roof is formed under which one may, by maneuvering, find a way through, because the shade is so dense that practically no new plants are able to gain a foothold. If the weight of the roof becomes so great that the shrubs or small trees supporting it are broken, then the *Dinochloa* roof in places rests on the ground. In this condition it is so jungle-like that it is very nearly impossible to penetrate without the use of a knife. Especially is this the case when growing with the *Dinochloa* there is a climbing palm (*Calamus*). All through the *Bambusa-Parkia* formation *Calamus mollis major*,[26] called "uay" by the natives, is found scattered. Especially when relieved of its support, portions of it lie recumbent or are intertwined among the branches of the *Dinochloa*.

The north ridge of the reserve from the sea coast to a distance of 2 kilometers inland shows the best development of this climbing bamboo. Here isolated trees of the *Bambusa-Parkia* formation, especially *Parkia, Albizzia,* and a few clumps of *Bambusa lumanpao*, are found. Some places show the entire absence of the *Dinochloa* and simulate the more shrubby parang to be described below. The species accompanying *Dinochloa* are practically the same as those found in the other types of parang, excepting that they are more scattered where the *Dinochloa* is thickest. Indeed, no continuous stretches of the *Dinochloa* parang prevail, but, rather, patches of it are encountered alternating with areas of other types. There is no doubt that the *Dinochloa* parang is found in places where artificial conditions have not been made. In that case

[26] The species of the genus *Calamus* known as rattan or climbing palms go under the Spanish name "bejuco" by the natives, who have individual names for the different species. Because of their economic importance the natives are especially keen in distinguishing them. Many of the species have not been scientifically determined and such species will be designated by their common names with the understanding that these names have no general application, for they may differ in the various provinces.

it is to be considered a natural and not an artificial stage in the development of the forest.

Streblus-Aphananthe Parang.—In order to give an idea of the floristic composition of other types of the parang, the following three plots are tabulated. Only trees which are over 4 meters in height are enumerated. Smaller species will be mentioned in a detailed description. The area of each plot is approximately 160 square meters.

DESCRIPTION OF PLOTS.

Plot A is on an old river terrace, 3½ kilometers inland and at an altitude of 70 meters. It has a gentle slope toward the river. Plot B is in a similar situation at 2½ kilometers inland and at an altitude of 50 meters; plot C is 2 kilometers inland and at an altitude of 35 meters.

TABLE XII.

Name.	A.	B.	C.	Total.
Streblus asper	6	6	4	16
Aphananthe philippinensis	8	1	5	14
Canarium villosum	3	5	2	10
Memecylon edule	2	7	1	10
Buchanania florida	4	3		7
Anisoptera vidaliana	7			7
Linociera cumingiana	4	3		7
Oroxylum indicum	1	1	3	5
Premna nauseosa	1	4		5
Zizyphus trinervis		4	1	5
Ficus haulii	1		3	4
Harpullia arborea			4	4
Lagerstrœmia speciosa			4	4
Pittosporum pentandrum	2	2		4
Polyalthia lanceolata		4		4
Ficus sinuosa			3	3
Macaranga bicolor			3	3
Parkia roxburghii	3			3
Semecarpus perrottetii	2	1		3
Mallotus philippinensis		2		2
Guioa perrottetii	1	1		2
Breynia acuminata	1	1		2
Artocarpus communis		1		1
Evodia glabra	1			1
Litsea tersa	1			1
Polysias nodosa	1			1
Shorea guiso	1			1
Terminalia nitens			1	1
Xylopia dehiscens		1		1
Total	50	47	34	131

A mere enumeration of the species does not give an adequate idea of this parang. Its profile is exceedingly irregular (Pl. IV). Individual specimens of the taller trees, *Anisoptera vidaliana, Macaranga bicolor, Parkia roxburghii, Buchanania florida, Artocarpus communis,*

and some times *Aphananthe philippinensis* overtop the others at varying heights. Disregarding the upper story, the younger trees together with mature smaller ones form a less regular profile. (See Table XII for an enumeration of the species.) Between the plots on the one hand and the "cogonales" on the other, more numerous in the tension zone or crowding in between the individuals of the lower story, are young trees or saplings of the older species and also the distinctly shrubby vegetation. *Tabernæmontana pandacaqui, Memecylon edule, Leea manillensis, Ixora cumingii, Breynia acuminata, Fluggea obovata, Micromelum pubescens, Guioa perrottetii, Streblus asper*, and *Premna nauseosa* are characteristic. By actual count, these predominate in the three plots under consideration; but the latter are varied so that the species which are found in one may or may not predominate in the others. The same may be said of the younger trees. For instance, in Plot B *Harpullia arborea* is represented by eighteen individuals, while it is not present in the other plots. In similar and even in adjoining patches of the type of parang under consideration, especially on the edges, there is found *Gmelina hystrix*, a small tree 3 to 4 meters in height, with long pendant branches, or *Mussænda grandiflora*, which accentuates its numerical importance by the large, white, bract-like calyx lobes. Among the smaller shrubs, the spiny *Plectronia peduncularis* is found throughout the different types of the parang, and *Otophora fruticosa* is but slightly less frequent. *Bridelia stipularis*, a common, subscandent shrub whose branches trail over the tops of the neighboring shrubs and small trees, has the appearance of a liana. The most common and large, woody lianas are *Uvaria rufa, Smilax bracteata, Tetracera sarmentosa, Ichnocarpus ovatifolius, Celastrus paniculatus, Cnestis ramiflora, Mucuna imbricata*, and the subscandent *Rourea multiflora*, all of which reach to the tops of the tall trees when these supports are present, or in their absence, sprawl over the tops of the lower story or even lie prostrate on the ground. Often *Dinochloa diffusa* (Pl. IV) and *Calamus mollis major* are found in this parang, which shows its close relation to the vegetation of the *Dinochloa* parang, which has already been described. Indeed, almost without exception, although their numerical importance is less, the species present in the former occur in the latter class. Another form of plant found in the parang is the smaller liana which differs from the larger one in habit as much as shrubs differ from trees. Among these the vitaceous *Cissus repens, C. japonica, C. geniculata*, and *Tetrastigma lanceolarium* are common; also there are species of other families such as *Abrus puchellus, Lygodium circinatum, Piper* sp., *Flagellaria indica*, etc. The herbaceous vegetation which appears to best advantage during the rainy season will be left to a later discussion.

Mixed parang.—In Plot A of the table given above it will be noticed that such species are present as *Anisoptera vidaliana, Shorea guiso*, and others characteristic of the *Bambusa-Parkia* formation. These trees give

character to the parang. Such a type is by Vidal[25] termed a "mixed" one in contradistinction to the parang proper, where species of the *Dipterocarpaceæ* are entirely absent. In Plot B a few seedlings of *Anisoptera vidaliana* and *Shorea guiso* were noted, although there were no mature trees, and in Plot C none at all were present. It should be noted that without exception the species found in all the different types of parang are present in the original *Bambusa-Parkia* formation, and in a discussion of the floristic composition of the latter, an enumeration of other than the large tree species was omitted to save needless repetition. It may be stated, then, that the mixed parang differs from the *Bambusa-Parkia* stage not in its floristic composition but in the numerical importance of the species of which it is composed. In the parang the smaller trees and shrubs predominate over the tall bamboo and the larger dicotyledinous trees, because the shade which formerly prevented their best development, had been removed. Indeed, as in the case of the *Dinochloa* parang, isolated patches of original vegetation very much like the parang proper or the mixed one may be found in the midst of the best development of the *Bambusa-Parkia* formation, although these are only encountered in especially dry, physiographic situations. The explanation of the *Dipterocarpaceæ* in Plot A perhaps lies in the fact that the original vegetation was not destroyed as completely as that in B and C, where not only are the *Dipterocarpaceæ* absent (except seedlings) but where the total number of larger trees is less. (See Table XII.) It might be maintained that the habitats which I have designated as Plots B and C are not able to support the vegetation of A, if it were not for the fact that in closely adjoining areas, in similar habitats, the *Bambusa-Parkia* combination prevails.

Other types of a parang occur over large areas nearer the seacoast on the south side of the reserve. They extend back to a distance of 4 or 5 kilometers and in places on the north side reach an altitude of 100 meters. These show a still greater reduction of the original vegetation, before the present growth was allowed to develop. According to the nature of the reduction there are three forms of this type. They are as follows: (1) Areas which have been reduced to a greater extent than those I have discussed, so that nearly all of the original tall trees have been cut out; (2) areas in which all the trees have been cut, but not all killed; (3) areas in which all of the vegetation has been removed, the land cultivated, and then the vegetation allowed to reëstablish itself naturally. This last form, which is the "caingin," may or may not pass through the grass stage before it reaches the arboreal one.

As previously mentioned, during the inroads on the forest, the best of the bamboo and *dicotyledinous* trees are taken out first. As the cuttings cause the timber which is most desired, to retreat farther from the shore

[25] *l. c.*, 10.

line, the difficulty of transporting the material taken out increases. After this period the less desirable timbers are removed. In this way all the taller trees may be destroyed and only those which are able to sprout from the stools will remain. In the meantime, the shrubs, vines, and worthless small trees which give character to the landscape have been given an opportunity subsequently to gain a better foothold. Even these may be removed partially for firewood, and as a result very low brush land is all which remains in the neighborhood of the towns. So various are the stages that it is difficult to classify them.

Largerstroemia-Zizyphus parang.—This form of parang has been named after *Lagerstrœmia speciosa* and *Zizyphus trinervis* because these two species predominate or at least give character to the landscape. I believe that both of them owe their place in this type to their persistent habit of sprouting from the base of the trunk after the tree has been cut. In this case they form low, shrubby trees which, with the other associated species, compose the type. In addition to the two tree species mentioned, in one plot of approximately 160 square meters, the following are common: *Bridelia stipularis, Premna nauseosa, Leea manillensis, Ficus hauili, Oroxylum indicum, Albizzia procera, Cordia blancoi, Harpullia arborea, Litsea tersa, Semecarpus perrottetii, Ficus sinuosa, Streblus asper, Canarium villosum, Tabernamontana pandacaqui, Otophora fruticosa.* With the exception of *Oroxylum indicum* and *Albizzia procera,* there is a tendency for this type to form an almost level top which is about 3 meters in height and which is thickly covered with herbaceous and woody vines; thus a dense shade is formed because of which, species not already present are practically excluded. However, *Oroxylum indicum* and *Albizzia procera* stand isolated above this raised platform and during the dry season their bare limbs are in striking contrast to the evergreen vegetation below. In other places *Polysias nodosa,* with a rosette of large pinnate leaves, rises to a height which is twice or thrice that of the green lower story.

Each partial attempt to clear such vegetation, or any further inroads made upon it to secure firewood and construction timbers of the lowest grade, tends only still further to reduce the coppice vegetation, or it gives greater opportunity for the growth of species like *Bridelia stipularis* and lianas of various sorts. In this way, just as a closely cropped lawn increases in thickness, the parang gains in density and loses in height.

It will be seen from the above reduction series that all stages of the parang may be obtained from the climax formation of the *Bambusa-Parkia formation* to that of the low *Lagerstrœmia-Zizyphus.* Many intermediate conditions were studied. In some places *Premna nauseosa* or *Gmelina hystrix* give a different floristic composition to the parang; but whatever the species, the ecological type is the same, namely, the coppice form, mixed with small trees having scandent branches and many small woody vines. The species which are thus reduced invariably reach a good

height if they are given an opportunity to develop and they are always found as taller trees in the bamboo forests or in more xerophytic physiographic situations within that forest.

The interpretation of the genetic degeneration of the *Bambusa-Parkia* formation given above is based almost entirely on comparative observation. Different stages from the lowest to the highest type may be seen lying adjacent to each other in a single habitat. These places usually adjoin the more reduced stages, viz, the cultivated clearings and cogonal. In two instances the progress of the vegetation on actual cuttings was observed at intervals throughout a period of fourteen months. Both, abandoned before completion, were made in the *Streblus-Aphananthe* type with the evident intention of clearing the land for cultivation. Many of the larger trees were left and in some places in the same clearing only slight inroads were made on the standing vegetation. An observation of these stages at later intervals, showed an incomplete development of all the forms of the parang just described.

It will be noted that no mention has been made of the vegetation occupying *total* clearings. It has been seen how the forest gradually has been reduced to the parang stage. It is only one step further to destroy it entirely. In the case of the parangs discussed, the forest is not cleared and prepared for cultivation; while in that of clearings the whole of the vegetation may be removed, the land tilled for a number of years and then abandoned, or what is more often the case, patches of it may be denuded, or large trees left standing. It will at once be seen that the resultant nature of the vegetation which invades these clearings when they are once more left to nature depends on their condition of reduction. Let it be assumed that the reduction is complete. Grass lands may then predominate in such clearings and a discussion of the causes of their development will first be given.

Cogonales.—Clearings in the Philippine forests or on their borders, which are covered by grasses, are known as "cogonales" (Pl. V), but there are no places on the Lamao forest reserve where the latter appear as the climax vegetation. Without exception, they are only stages in the reëstablishment of the normal vegetation removed by man. The first periods in the development of vegetation in caingins, in the best expression of the *Bambusa-Parkia* forest, have not come under my observation, but it is not thought that they differ materially from those in the parang after it has been well established or indeed in clearings made in the *Anisoptera-Strombosia* forests to be discussed below. Usually, such clearings are made on the borders of others and consequently, when abandoned, many seeds from these places find early lodgment. However, if the clearing is made during the dry season the migrated seeds do not develop until the wet one. This gives an opportunity for seeds to migrate before many germinate and therefore the first season shows vigorous weeds such as *Blumea balsamifera*, *Elephantopus mollis*, *Emilia flammea*,

E. sonchifolia, Adenostemma viscosum, Ageratum conyzoides, Triumfetta rhomboidea, Urena sinuata, Scoparia dulcis, Abutilon indicum, Sida carpinifolia, Commelina nudiflora, Solanum ferox, Hyptis suaveolens, H. spicigera, Euphorbia pilulifera, and others. Among the vines are *Momordica charantia, Merremia hastata,* several species of *Ipomœa* and others. All are more or less rapidly growing, herbaceous forms with seeds adapted to easy distribution. During the dry season many of the above have partially disappeared.[27] Those which are left show a reduction of leaf surface, so that the thinness of the vegetation in these first stages is comparable with that of the tree vegetation of the *Bambusa-Parkia* formation. At this time a number of grasses also find a place. Among them may be mentioned *Panicum pilipes, P. sarmentosum, Oplismenus burmannii,* and *Centotheca lappacea. Imperata exaltata,* the principal cogon grass, gains an entrance more slowly, but after the first year it begins to give a decided aspect to the vegetation. This is due to the rapid development of underground shoots by means of which, from a common center, it can migrate in all directions, and soon establish a more or less dense sod which shades out other forms. With the appearance of the cogon there is initiated a new period which may be prolonged to a greater or less extent. If the soil is shallow, as is often the case on an exposed ridge where erosion is great, many of the more mesophytic forms, growing with the grass during the wet season, may not be able to survive the period of drought, and thus the encroachment of woody forms is slow. Nevertheless, if the periphery of the "cogonal" is the edge of a wooded area, the shade on the border may be sufficiently dense to make impossible the existence of the grass, although woody species which are not so light-demanding, may obtain a foothold. With each generation the shady periphery advances on the island of grass and in time replaces it.

Again, in more mesophytic places, a grass land once established offers a more or less favorable feeding place for cattle, carabaos, and horses. In order that the coarse grass may not become too woody, or for other reasons, the owners may burn the area periodically. Thus many ligneous species which may have started are killed, while the geophilous parts of the grass are uninjured. Hence, by repeated fires, cogonales are maintained indefinitely and even enlarged at the expense of the neighboring woody formation. As soon as the fires are checked, the invasion begins anew and in time the grass will be crowded out. The rapidity of the invasion of woody species depends on the nature of the surrounding

[27] The line between annual and perennial on the one hand and herbaceous and woody plants on the other can not be so closely drawn in the Tropics as in temperate regions. The life of the same plant may be prolonged in one situation over the dry season, while in others, less favorable, it dies down to the ground. Most of the plants mentioned in this list can spring from seed, and flower in one season. Usually the rapid growth is during the rainy season while at its close, reproductive activity sets in. Most of those having perennial root stalks become more or less woody and persistent and therefore can not strictly be called herbs.

vegetation and on the chresard of the soil. If the chresard is high, it will be rapid; if, as indicated above, it is low, then it will be slow. *Imperata exaltata* grows to the height of a meter or more. This altitude together with its more or less dense sod enables it successfully to compete with other grasses, but occasionally openings in the "parang" are found in which *Andropogon aciculatus*, which is propably a better forage grass than cogon, is present; this forms a carpet, but unlike the cogon it spreads by runners above ground instead of by root stalks. It does not grow to a great height and many instances were noted where it is being crowded out by the cogon. The *Andropogon* areas are never so extensively developed as the cogonales although patches of the former are often found in the latter. *Imperata arundinacea koenigii*, the smaller "cogon" grass, is also present in the grass lands, although it, like the *Andropogon* is not so successful as *I. exaltata.* While many other grasses are present, especially in the early stages of the development of the "cogonales," yet none are conspicuous numerically. The large bunch grass, *Saccharum spontaneum*, so characteristic of the lowlands in semi-hydrophytic conditions, is confined to individual specimens on the rocky flood plains of the rivers and to the abandoned rice lands near the mouth of the Alangan River.

Psidium parang.—The invasion into the cogonales of arboreal species, or into caingins, brings about the initial stages of temporary formation in which tree instead of grass species predominate. After the beginning "weed" stage the cogonal, as shown above, may intervene before the tree stage is reached, or if the conditions necessary for the cogonales are not obtained, the transition may be more or less direct. All the steps in the transition from the newly made caingin to the fully developed woody type have been observed. If the caingin is surrounded by a forest in which there are no exotic species, the seeds of the latter which enter will need to be blown by the wind or carried a long distance by birds and other animals. The chances are that none or but a few exotics will be present. Without exception, clearings observed in the *Anisoptera-Strombosia* forest had no exotic tree species. The surrounding forest or the closely lying open places furnish the seeds which are to develop into the plants which form the invading vegetation. The floristic composition will be discussed under a treatment of such clearings in connection with the *Anisoptera-Strombosia* formation.

As in the case of cuttings, the first "clearings" are made near the seacoast, or if inland, they may radiate from a common center (settlement) in one or more directions. When, finally, the clearing has been made and abandoned, as already described, it may pass through the cogonal stage; but if the conditions for the development of the latter are not present, then the arboreal one has an early beginning. The most common tree species found in these clearings and the first one to enter is

Psidium guajava (guava.) Blanco[28] to the contrary notwithstanding, this plant is believed to have been introduced from Mexico, its original home. The facts that it has no native name and that it is never found where the original vegetation has been undisturbed, certainly constitute strong evidence in favor of its exotic nature. The guava is well fitted for distribution. The edible, fleshy fruit is well filled with very small seeds which, when eaten by hogs, birds, etc., pass through the intestines undigested. In this way they may gain an early entrance into abandoned clearings. They are even found scattered through farms which are still in a state of cultivation. Such clearings early come to have a "guava" aspect. The tree sprouts freely, and if it is cut for fuel, new shoots are produced. Types of parang, which show no places where the vegetation has entirely been removed do not have the guava, but a partial removal of the vegetation intermixes the other types with that of the guava. Other species will also soon invade clearings from which all the vegetation has been removed, and then will join the guava in forming the new growth. In this way large tracts of land have come to be occupied by the guava mixed with the species of the other parangs. On the reserve there are all stages in the development of clearings. An enumeration of the species will be only a repetition of those already given. Groups of other cultivated trees which show the former condition are scattered through the guava parang. Thus isolated individuals or groups of *Mangifera indica* (mango), *Anacardium occidentale* (cashew), *Artocarpus integrifolia* (jack fruit), bananas, papaya, *Ceiba pentandra, Areca catechu,* and in one case *Cocos nucifera* were found scattered through the various stages in the development of a parang. Mention has already been made of the place in the vegetation of the building bamboo (*Bambusa blumeana*). No attempt has been made to enumerate all the indigenous species which were found. In spite of the detailed description of the different types, it is still felt that justice has not been done to the extreme floristic complexity of the vegetation.

Seasonal changes of the formation.—The aspect of the parang, like that of the *Bambusa-Parkia* forest, changes with the season. During the dry one many of the herbs die to the ground and all are much reduced in foliage. The trees show the result of drought and drop a portion of their foliage.

As a whole, the transpiration surface is decidedly thinner. While some of the trees flower irrespectively of season, yet the reproductive activity of a much larger number begins in May and continues through July. These three months are distinctly the flowering ones. Vegetative ac-

[28] Blanco, M., Flora de Filipinas (1845), 292, claims that fossil forms of this plant have been found by the natives in volcanic rocks near Manila. He does not state that he has seen these plants himself. Brandis (See Forest Flora of India, 232) and others claim that it is certainly exotic in India and the Malayian region in general.

tivity may be present in all, but it is decidedly much more in evidence throughout the entire length of the wet season. During 3 or 4 months bamboo culms reach a height of from 12 to 15 meters, the shoots of trees develop rapidly, and herbaceous vegetation springs up with surprising quickness, the change from the dry to the wet season resembling to some extent the spring activity of vegetation in temperate regions.[29] Aside from the clothing of the trees with their full quota of leaves, the appearance of numerous herbs of various kinds decidedly changes the aspect of the vegetation. However, it is strikingly true that almost no herbs appear in the dense parang growth or in the bamboo forests, save here and there a few ferns, but on the edges of the clearings, in cogonales and, indeed, in any place where the vegetation is sufficiently thin to admit light to the ground, the latter is soon covered with various herbs. Among these, weeds such as *Hyptis, Triumfetta, Adenostemma,* etc. (see p. 396), are apt to form thickets on the edge of cogonales, where the shade of the surrounding tree vegetation is too great for the grasses. Among the first geophilous herbs is *Amorphophallus campanulatus.* About the middle of May, in places, in the thinner parts of the parang this species develops from a spherical corm, 15 to 20 centimeters in diameter, to an inflated spathe as large as one's head. Soon after the flower dies, a three-parted leaf with the segments pinnatified and with a spotted petiole appears. Often this reaches the height of 2 meters and is strikingly prominent in contrast with the surrounding vegetation. About the same time the flowers of *Curcuma zedoaria* and *Zingiber zerumbet* become apparent, to be followed by colonies of the canna-like leaves of the *Curcuma* and the pinnate ones of *Zingiber.* These are the most prominent of the geophilous herbs which frequently occupy large patches to the exclusion of all others. Cogonals which are brown in the dry season, rapidly turn green during the wet one, and in places where the grass is thin, many herbaceous plants or seedlings of arboreal species appear. In July, colonies of *Ophioglossum nudicaule,* or single individuals of *Helminthostachys zeylanica* among the ferns, grow in half open places on the edges of the parang or of the bamboo. By September these have disappeared. Prominent ferns, all but geophilous during the dry season, are *Hemionitis arifolia, H. gymnopterodea, Gymnopteris taccifolia, Polybotria apiifolia* and *Adiantum philippense. Cheilanthes tenuifolia* is xerophytic and tropophilous. *Anthoceros grandis* of the liverworts, prominent during the wet season on moist banks, and a few mosses are all which were noted of the *Bryophytes.*

Epiphytes.—The epiphytic vegetation, although not well studied, shows a scarcity of species in comparison with the formations higher up

[29] However, it must not be overlooked that many trees show vegetative as well as reproductive activity during the period of low chresard. This is especially the case with plants growing on the edges of water courses, where the chresard is more uniform throughout the year.

the mountain. The evergreen species of the parasitic *Loranthaceæ* which are found on the limbs of *Parkia roxburghii* (Pl. III) and on other species are, however, very striking. These are prominent features of the sky line when the foliage of the trees is thin. Very common also, in open places, on the trunks of trees, on rocks, and on fallen logs, is the deciduous epiphytic fern, *Drynaria quercifolia.* During the favorable season it has dimorphic leaves. During the dry season, the foliage leaf is shed and the scale leaf becomes dry and brown, strikingly showing the effect of the change of seasons on the formation in which it grows. A few epiphytic orchids were noted and were collected, but as the species as yet have not been determined the list can not be given here. The twining asclepidaceous epiphytic *Dischidia pectenoides* is usually found growing on bamboo near the upper limits of the formation. Besides the ordinary leaves, it has bladder-like ones which generally harbor insects and in which delicate roots are developed.

Relation of the vegetation to the topography.—In the preceding discussion some mention has been made of the varying types of vegetation in different physiographic situations. With the exception of the land lying close to the river, the physiographic types are ridges, slopes of ridges and old river terraces at or near their bases. Except when the latter are of such recent origin that the underground water level is near the surface, they do not offer any strikingly different habitat for plants than the more gentle slopes. The terrace appearance is often destroyed by the surface wash of the slopes and thus it grades imperceptibly into the latter and becomes a part of it. The river is young, so that as yet there have not been developed any flood plains, although during the rainy season the lowest terraces are flooded. The vegetation of these and of the river channel itself will be considered in another place. On the exposed ridges the vegetation is slightly more xerophytic than it is on the slopes, and here its composition is similar to that of the parang.

Future possibilities of the parang vegetation.—Since the parang is not peculiar to the Lamao Reserve, but is found throughout the Philippines, it is of considerable importance to ascertain what its forestal and agricultural possibilities are, and whether, in case it is left idle, it will again return to its normal condition. As long ago as 1880 Vidal [30] called attention to the idle nature of these lands and then he advocated their agricultural possibilities. No doubt much of the land is available for agriculture, but that portion which is situated on the slopes is better suited for purposes of forestry. Outside the reserve, on many mountain sides, the parang or cogonales extend well up on slopes which formerly were covered with virgin forests. The latter acted as better retainers of moisture than does the present vegetation and with the advent of a more intense agriculture at their bases, if they are returned to their original

[30] L. c., p. 10.

condition, they will be not only the future source of a timber supply but they will be the best conservers of water for irrigating purposes.

There is evidence to show that without the aid of man much of the "mixed" parang of the Lamao Reserve is returning to the original condition of the dicotyledinous portion of the *Bambusa-Parkia* formation. However, this does not include the bamboo portion of the formation. To understand the reason for this, it is necessary to know something of the seeding habits of this class of plants. It is obvious that, vegetatively, the "bunch" bamboos can not spread in such a manner as to occupy new territory, therefore, this type is wholly dependent on seeds for its distribution. With the exception of the climbing bamboo, *Dinochloa*, none of the bamboos of the Philippines have been collected in seed, and therefore it is apparent that the capacity of the "bunch" bamboos to spread from seed has not come under observation. The evidence furnished by British Indian foresters throws some light on the reproductive habits of this variety of vegetation. Kurz[31] is authority for the statement that a number of bamboos flower simultaneously after a lapse of years and then die. Brandis[32] states that isolated flowering clumps of *Bambusa arundinacea* are occasionally found, but as a rule, clumps in one flowering district come under flower simultaneously while a few may be found earlier and some in the following year. This author[33] further says that *Bambusa polymorpha* in 1859 flowered and then died, in a forest of this bamboo found in Pegu under tall trees of *Xylia dolabriformis, Terminalia tomentosa, Tectona grandis* (teak), and others. In 1861 there were many millions of seedlings. Only when a year of bamboo seeding takes place can suppressed young teak obtain a foothold or do teak seedlings appear. While there is no authentic record of the bamboo of the Lamao Forest Reserve or any of the common clump species of the Philippines having flowered yet, according to the authority of the natives, these species behave in much the same manner as do those described by Brandis and other Indian foresters. It may not be out of place to predict that in case the bamboo called "boho" by the natives, flowers simultaneously at more or less regular periods, then there may be a decided subsequent change in the aspect of the *Bambusa-Parkia* forests. If the older stalks die down at the time of the flowering of these bamboos, then the park-like nature of the dicotyledinous growth will become very apparent. Practically, then, there will be a new habitat to which light will have access. The suppressed dicotyledinous trees will spring up more rapidly, the ground will be freely occupied by *Bambusa* seedlings and by the sprouts of dicotyledinous trees which had seeds available at the time of the death of the bamboo. Then a struggle will begin between the seedlings of various kinds and the saplings and young

[31] Curz, S., *Forest Flora of British Burma*, 1: (1877) Introd., p. XXIX.

[32] Brandis, D., Flowering of *Bambusa arundinacea Ind. For.* 13: 509.

[33] Biological Notes on Indian Bamboos. *Ind. For.* (1899) 25: 1 to 25.

trees already present. Many of these latter no doubt will hold their own and will occupy a place in the new, matured forest which will be developed. Owing to the rapidity of the growth of the bamboo seedlings, but few of the dicotyledinous seedlings will be able to compete successfully with them. Attention also should be called to the fact that according to the observations of Indian foresters, from the seed it takes from eight to ten years for the culms of *Bambusa arundinacea* to reach full size. No doubt a number of years would elapse before the culms of "boho" could reach their ultimate height of from 15 to 18 meters. If such be the case, this period of maturity would be sufficiently long to permit the young trees and saplings to gain considerable headway in a young forest of bamboo. Thus, as the bamboo growth reaches maturity, the dicotyledinous trees present, but which previously had been suppressed, will also reach maturity and the new forest which is thus developed will in many respects resemble the original one which it replaces. Kurz[34] is also authority for the statement that where bamboo growth in the original forest has taken place around clearings or what would correspond to cogonales, these bamboo seedlings soon spring up and choke out all other vegetation except that of light-loving, quick-growing sapling trees. If this should become the case on the Lamao Forest Reserve, then portions at least of the cogonales will be captured by the bamboo growth. Whether the bamboo seedlings will be able successfully to survive or even whether the seeds will germinate at all under the tolerable dense shade of the forest types of parang growth, remains to be demonstrated. It is not likely that many seedlings will be able to exist under such conditions. It must be understood that what is said represents only a conjecture. Extensive observation on the spot is needed to demonstrate the actual behavior of the bamboo when it seeds.

As I have already mentioned, there is evidence to show that the dicotyledinous trees of the original growth of the *Bambusa-Parkia* formation are gradually encroaching on the parang, especially at its thinner portions and into the cogonales themselves. Many instances were noted where seedlings were starting in the semi-shaded area produced by the woody growth of the periphery of the cogonales. Where the parang borders on the grass areas, then the species which are thus introduced are rather more generally those of the parang than of the original growth. Nevertheless, if the parang is in the neighborhood of seeding trees of the latter class, or if these trees be in the parang itself, there will be developed some seedlings. After a number of years at least some of the latter will overtop the low growth of the parang, causing it to become more or less suppressed, and it therefore will be reduced to a subordinate place in the new forest thus established. Many instances were noted where such alterations were taking place. Of course, the new forest would almost entirely be a dicotyledinous one unless, during some

[34] L. c., Introd., p. xxx.

stage of its development, the bamboos in the neighborhood should seed and find an entrance into it. The areas of the parang situated at some distance from the original forest would in all likelihood not be stocked with seeds from the former and thus the forest in these places would need to pass through a number of generations of growth, each successive one approaching nearer to these distant areas, before the latter could be reclaimed.

Summary.—1. The *Bambusa-Parkia* formation occupies the region of the Lamao Forest Reserve at the base of the mountain. It is divided into two types, i. e., the climax and the parang. The former type occupies the upper portion of the formation, but has islands lower down and peninsulas extending nearly to the sea. Its area is approximately one-third of the entire formation. The latter has tongues extending into the former. It occupies approximately two-thirds of the entire formation. In this type are included the cogonales.

2. All species in the parang except *Psidium guajava* are represented by suppressed forms in the climax type, or they are prominent forms which are there reduced to the coppice condition. Xerophytic areas of the climax type (like exposed ridges or ends of ridges) resemble parang forms.

3. If left undisturbed, parang types will gradually return to the climax, with or without the bamboo.

4. The parang type includes:

Cogonales.
Psidium parang.
Dinochloa parang.
Lagerstrœmia-Zizyphus parang.
Streblus-Aphananthe parang.
Mixed parang.

5. The climax forest is closely related to Schimper's [35] monsoon tropical forest or Kurz's [36] mixed tropical forest.

6. The *Bambusa-Parkia* formation, in contrast with the forest higher up the slopes of the mountain, is more xerophytic. This is due to less rainfall, lower relative humidity and greater heat.

7. The xerophytic nature of this formation finds expression in deciduous and partially deciduous trees, in semi-xerophytic structures, evergreen trees, and in geophilous or semi-geophilous herbs.

II. Anisoptera-Strombosia Formation.

This formation lies immediately above the *Bambusa-Parkia* forest. *Anisoptera vidaliana* (mayapis) is representative of the largest tree species in which *Dipterocarpaceæ* predominate. *Strombosia philippinensis* is the principal tree of the second story and stands for a group

[35] L. c., 352.
[36] L. c. Introd., p. XXV.

having a place in this forest similar to that occupied by the bamboo in the *Bambusa-Parkia* formation. Indeed, on the border between the two there is a mixture of this element with the bamboo; for both together, or islands of one surrounded by the other, prevail as a second-story vegetation.

FORESTS OF RIVER TERRACES.

Much has been written concerning the floristic composition of a tropical forest. Lists of tree species have been made which show the number of species, but so far as is known there exists no quantitative analysis of a tropical forest.[37] The following tables will give some idea of the complexity of the forests of the Lamao Reserve.

Description of Table XIII.

The plot is on a low, rocky, narrow terrace of Lamao River at an altitude of 100 meters. It comprises an area of approximately 8,000 square meters. A gives the number of trees over 4 meters in height; second line under this heading, the number under 4 meters in height, including seedlings; B, the percentage of each species, and C, the percentage of trees of each species in the adjoining hillside plot. (See next table.)

TABLE XIII.

Name of plant.	A.	B.	C.
1. Anisoptera vidaliana	28 25	8.3	2.10
2. Mangifera altissima	24 35	7.0	.11
3. Lagerstroemia speciosa	14	4.1	.22
4. Eugenia luzonensis	11 7	3.3	
5. Palaquium luzoniense	11 2	3.3	1.22
6. Calophyllum wallichianum	10 8	2.9	.77
7. Phœanthus cumingii	9 8	2.6	1.00
8. Bischofia trifoliata	8	2.31	
9. Canarium villosum	8 2	2.31	
10. Gnetum gnenom	8 4	2.31	7.74
11. Diospyros pilosanthera	7 26	2.02	4.64
12. Pandanus luzonensis	7 Many.	2.02	.44
13. Parkia roxburghii	7 Many.	2.02	.55
14. Pithecolobium acle	7 2	2.02	
15. Dysoxylum cumingianum	6 6	1.73	.11
16. Baccaurea tetrandra	6 10	1.73	1.19
17. Koordersiodendron pinnatum	6 14	1.73	1.11

[37] The writer has not had access to all the literature on the subject.

TABLE XIII—Continued.

Name of plant.	A.	B.	C.
18. Leea manillensis	6 40	1.73	
19. Reinwardtiodendron merrillii	6 43	1.73	3.21
20. Dipterocarpus vernicifluus	5 12	1.44	2.21
21. Shorea contorta	5	1.44	2.65
22. Zizyphus zonulatus	4	1.15	
23. Cratoxylon blancoi	4 1	1.15	
24. Dracontomelum cumingianum	4 1	1.15	.83
25. Knema heterophylla	4 3	1.15	1.11
26. Memecylon edule	4 7	1.15	1.11
27. Shorea guiso	4 6	1.15	3.43
28. Strombosia philippinensis	4 13	1.15	10.50
29. Tabernæmontana pandacaqui	4 14	1.15	.44
30. Celtis philippinensis	3 1	.86	.88
31. Cyclostomon bordenii	3 10	.86	2.88
32. Cynometra simplicifolia	3 1	.86	.33
33. Dracæna angustifolia	3 11	.86	.22
34. Ellipanthus luzoniensis	3	.86	.66
35. Eugenia bordenii	3 6	.86	.55
36. Ficus hauili	3 4	.86	.44
37. Kayea paniculata	3 2	.86	
38. Kleinhofia hospita	3	.86	
39. Pterospermum niveum	3 5	.86	
40. Radermachera banaibana	3	.86	.22
41. Sarcocephalus cordatus	3	.86	
42. Turpinia pomifera	3	.86	2.10
43. Cryptocarya luzoniensis	3 5	.86	1.00
44. Linociera coriacea	3	.86	.55
45. Aglaia harmsiana	2	.57	2.32
46. Artocarpus communis	2	.57	.11
47. Champereia cumingiana	2	.57	1.44
48. Chisocheton tetrapetalus	2	.57	1.33
49. Cyathocalyx globosus	2	.57	.55
50. Cyclostemon microphyllus	2	.57	.11

TABLE XIII—Continued.

Name of plant.	A.	B.	C.
51. Eugenia sp	2	.57	
52. Euphoria cinerea	2	.57	1.88
53. Ficus caulocarpa	2	.57	
54. Glochidion album	2	.57	
55. Grewia stylocarpa	2	.57	.77
56. Leea philippinensis	2	.57	.55
57. Terminalia nitens	2	.57	
58. Sp. indet	2	.57	.11
59. Aglaia turczaninowii	1	.29	
60. Aphananthe philippinensis	1	.29	.66
61. Artocarpus lanceolata	1	.29	.33
62. Buchanania florida	1	.29	.22
63. Canangium odoratum	1	.29	
64. Casearia fuliginosa	1	.29	.44
65. Citrus hystrix	1	.29	
66. Elæocarpus oblongus	1	.29	
67. Eugenia acuminatissima	1	.29	
68. Ficus pseudopalma	1	.29	
69. Ficus paucinervia	1	.29	
70. Ficus sp	1	.29	
71. Ficus sp	1	.29	
72. Ficus sp	1	.29	
73. Garcinia binucao	1	.29	.55
74. Guioa perrottetii	1	.29	
75. Leucosyke capitellata	1	.29	
76. Neolitsea vidalii	1	.29	.11
77. Macaranga tanarius	1	.29	
78. Myristica philippensis	1	.29	.66
79. Palaquium tenuipetiolatum	1	.29	1.22
80. Palaquium whitfordii	1	.29	
81. Polyalthia barnesii	1	.29	.66
82. Polyscias nodosa	1	.29	.22
83. Pterospermum diversifolium	1	.29	.33

TABLE XIII—Continued.

Name of plant.	A.	B.	C.
84. Pterospermum obliquum	1	.29	.44
85. Pygeum latifolium	1	.29	.22
86. Sideroxylon macranthum	1	.29	.22
87. Spondias mangifera	1	.29	
88. Talauma villariana	1	.29	1.19
89. Xanthophyllum sp	1	.29	1.66
90. Sp. indet	1	.29	
91. Sp. indet	1	.29	
92. Sp. indet	1	.29	
Total	328		

Relation of vegetation to topography.—As previously stated, the Lamao River is distinctly an eroding one. At the altitude of the above plot there is a fall of 7 in a total distance of 300 meters; at high water the stream floods some portions of the terrace and maintains one prominent channel through it. This leaves the terrace divided into two parts; one, which is made up of large boulders, is from 2 to 2½ meters above the river and stands between it and the flooded channel; the other, somewhat higher, lies at the base of the adjoining hill, and though it contains some boulders, yet these are more or less covered with surface wash from the slopes of the hill. A very small amount of silt is deposited between the rocks in extreme floods, when nearly all of the boulder terrace is under water for a short time. The absence of much soil and the periodic presence of a swift current make it a difficult place for plants to gain a foothold, consequently the vegetation is more or less open.

The wet-weather channel is more or less destitute of vegetation during the rainy season because of the swift currents periodically sweeping through it. During the dry one, many young plants of *Pandanus luzonensis, Eugenia luzonensis*, and others spring up, only to be to a great extent washed out during the following wet period. The vegetation on the boulder flood-plain is very much like that encountered in some portions of the *Bambusa-Parkia* formation. *Anisoptera vidaliana. Lagerstrœmia speciosa, Dracontomelum cumingianum, Parkia roxburghii, Cananium villosum*, and *Zizyphus zonulatus* are here present.

On the borders of the wet-weather channel and of the stream *Eugenia luzonensis, Bischofia trifoliata, Sarcocephalus cordatus*, and *Kleinhofia hospita* (Pl. VI) are characteristic and, so far as the Lamao Reserve is concerned, are found in no other ecological situation. The terrace at the foot of the hill shows a condition similar to that on the adjoining

slopes. A reference to the table will make apparent the relative proportion of species to be found in the entire plot and on that of the hillside. *Mangifera altissima* is the only species on the less boulder-like terrace which is not also common on the hillside.

Because of the park-like nature of the entire terrace, the underbrush is here more common than it is in the more closed forest about to be described. The shrubby species which are common and characteristic of the river bottom are *Lunasia amara, Justicia gendarussa, Glochidion sp.*, many seedlings of *Pandanus luzonensis*, jungle-like thickets of *Donax arundastrum*, and a few clumps of *Bambusa lumanpao*. Among the herbaceous forms are *Hypœstis cinerea*, *Clerodendron intermedium*, and *Alpinia brevilabris*. The following ferns are also found here: *Microlepia speluncæ*, *Gymnopteris contaminans*, *Lygodium circinatum* *Polypodium myriocarpum*, growing on rocks and on the base of trees, and *Pteris cretica*. A number of grasses are common in the open places.

Epiphytes.—The epiphytic vegetation is probably more prominent than observations show. The difficulty of obtaining a good idea of the character of the tops of the trees is great. However, a number of epiphytic orchids were obtained from tall trees by the natives and some were found on low branches in places having sufficient light. These include five species only. The epiphytic fern *Drynaria quercifolia* grows in open places on the trunks or branches of trees.

Lianas.—The liana vegetation is very characteristic. Because of the excess of light, the vines are encountered not only in the tops of the trees but in places they even form dense walls on the open sides of the more closed areas. *Symphorema luzonicum* is perhaps the most characteristic of this vegetation and its habit may be taken as a type of that of all of the larger vines. Growing as it does to the tops of the large trees, by its own weight it may drag lower portions of its own length to the ground so that it may lie recumbent. From these parts in the denser shade new shoots may arise. These often remain suppressed until a natural opening will give them an opportunity for rapid development. Thus the new shoots may trail to a greater or less extent until a support is reached, after which they become erect and in time will grow to the tops of the trees. Often, the prostrate portion becomes covered with earth, so that the shoots arising from it resemble young trees starting in the dense shade of the forest. During the wet season and growing in the dense shade, the long, etiolated, leafless shoots of the *Gnetum latifolium* (another common liana) resemble saprophytic plants so nearly that one is easily deceived concerning their true nature. Indeed, such shoots are in one sense saprophytic, for when they grow in a situation where the light is insufficient to enable them to elaborate their own food, they are dependent upon the foliage at the tops of the tall trees for their nourishment. Often three or more vines are found on a single tree. One instance emphasizing this fact was noted where *Symphorema*

luzonicum, Gnetum latifolium, Pothos philippinensis, Pothoidium lobbianum, Freycenetia luzonensis, and the large leaved *Conocephalus violaceus* were growing on the same individual. Among the other large lianas noted were *Strychnos multiflora, Tetracera sarmentosa, Dinochloa diffusa, Ficus bordenii, Anamirta cocculus, Combretum squamosum, Cnestis ramiflora,* five species of *Calamus, Stixis philippinensis, Rivea luzonensis,* species of *Cissus, Mucuna imbricata, Dioscorea pentaphylla,* and others. Some idea will be given of the character of the vegetation by the three photographs taken in or near the plot in consideration. (Pls. VI, VII, VIII.)

The character of the vegetation on terraces similar to that already described, but the boulders lying in which are to a greater extent covered by the wash of the adjoining slopes and which usually are slightly higher in situation, is shown by Plates IX, X, XI, and XII.

FORESTS OF SLOPES.

Description of Table XIV.

The plot classified in the following table is on the hillside, extending up the slopes at right angles to the one on the river valley terrace (See Table XIII). It is approximately 60 meters in width and 170 meters in length. The area thus contains 10,200 square meters. The gradient averages about 27 degrees, but in places it is as much as 40 degrees, and for a short distance halfway up the hill it is nearly level. The plot is divided into 5 subplots roughly corresponding to 15-meter contour lines. Beginning at A, the plot runs uphill to E. The column F gives the total number of trees over 4 meters in height in all the plots, and G the total number under 4 meters. H gives the percentage of each species, and I the percentage of species in the river terrace plot (see Table XIII).

TABLE XIV.

Name.	A.	B.	C.	D.	E.	F.	G.	H.	I.
1. Strombosia philippinensis	10	13	24	27	20	94		10.50	1.15
	9	13	45	13	33		113		
2. Gnetum gnemon	3	3	12	15	37	70		7.74	2.31
		4	3	2	9		18		
3. Diospyros pilosanthera	3	5	11	11	12	42		4.64	2.02
	8	8	20	25	24		80		
4. Aporosa symplocosifolia		1	5	2	30	38		4.20	
	1	4	5	3	15		28		
5. Shorea guiso	2	6	13	6	4	31		3.43	1.15
	1	1	30	24	20		76		
6. Reinwardtiodendron merrillii	8	14	5	2		29		3.21	1.73
	27	8	22	9	2		68		
7. Cyclostemon bordenii	6	6	8	3	3	26		2.88	.86
	8	21	16	19	9		73		
8. Shorea contorta	2	1	11	5	5	24		2.65	1.41
	3		4	3	2		12		
9. Aglaia harmsiana	8	6	2	4	6	21		2.32	.57
	2	5	6	8	14		35		
10. Dipterocarpus vernicifluus	4	3	11	1	1	20		2.21	1.44
	10	20	15	7			52		
11. Anisoptera vidaliana	1	2	4	8	4	19		2.10	8.30
		1	4	3	2		10		
12. Turpinia pomifera		1	1	9	8	19		2.10	.86
		1	1	2	3		7		

TABLE XIV—Continued.

Name.	A.	B.	C.	D.	E.	F.	G.	H.	I.
13. Baccaurea tetrandra		4	7	4	3	18		1.19	1.73
	3	2	14	15	6		40		
14. Talauma villariana		5	7	3	8	18		1.19	.29
		2	2	5			9		
15. Euphoria cinerea	2	3	6	3	3	17		1.88	.57
	10	8	5	11	9		43		
16. Xanthophyllum sp.	5	3	4	1	2	15		1.66	.29
		1	8	7	4		20		
17. Antidesma edule	1		10		2	18		1.44	
	2	1	3	3	1		10		
18. Champereia cumingiana	1		2	4	6	13		1.44	.57
			8		3		6		
19. Chisocheton tetrapetalus		4	4	3	1	12		1.33	.57
	1	12	1	5	5		24		
20. Atalantia disticha	1				10	11		1.22	
	1	1	1	2	35		40		
21. Palaquium luzoniense	1	2	1	4	3	11		1.22	3.30
	1		1	5	4		11		
22. Santiria nitida			3	1	7	11		1.22	
			2	3	3		8		
23. Palaquium tenuipetiolatum		1	6	3	1	11		1.22	.29
		2	2	1	2		7		
24. Dimorphocalyx longipes				2	9	11		1.22	
					10		10		
25. Memecylon edule		2	3	1	4	10		1.11	1.15
		4	7	3	27		41		
26. Knema heterophylla	1	2	2	2	3	10		1.11	1.15
	3	1	3	4			11		
27. Koordersiodendron pinnatum		1	4	1	4	10		1.11	1.73
		1	4	1	1		7		
28. Phaeanthus cumingii	5			1	3	9		1	2.60
	10	1	8	3	2		19		
29. Cryptocarya luzoniensis	3		3	1	2	9		1	.86
	1	1		2	6		10		
30. Celtis philippinensis	1		1	2	4	8		.88	.86
	2	6	9	2	5		24		
31. Mitrephora ferruginea	1	5		2		8		.88	
		3			1		4		
32. Calophyllum wallichianum	2	1	3	1		7		.77	2.90
	15	25	27	26	12		105		
33. Grewia stylocarpa	2	1	3	1		7		.77	.57
	2	7	4	11	5		29		
34. Xylopia dehiscens	1		2		3	6		.66	
			5	1	16		22		
35. Polyalthia barnesii			2	3	1	6		.66	.29
	2	4	5	5	1		17		
36. Ellipanthus luzoniensis		2	2	2		6		.66	.86
	4	1	3	1	2		11		
37. Macaranga mappa		4		1	1	6		.66	
		1			2		8		
38. Aphananthe philippinensis	4	1	1			6		.66	.29
39. Myristica philippensis	2			2	2	6		.66	.29
40. Garcinia binucao			1	3	1	5		.55	.29
	5	6	25	25	7		68		
41. Eugenia bordenii	2		2		1	5		.55	.86
	2	3	12	2	4		28		
42. Leea philippinensis	1	1		1	2	5		.55	.57
	1		1	4	2		8		
43. Linociera coriacea	1	1	1	1	1	5		.55	.86
	1	2	1	1	3		8		
44. Cyathocalyx globosus		1	3	1		5		.55	.57
		2	5				7		
45. Polyalthia flava			1	1	3	5		.55	
	1				1		2		

TABLE XIV—Continued.

Name.	A.	B.	C.	D.	E.	F.	G.	H.	I.
46. Parkia roxburghii	3	2 1				5	 1	.55	2.02
47. Alstonia scholaris		1	2	1	1	5		.55	
48. Hopea acuminata		1	2 2	1 1	1	5	 3	.55	
49. Tabernæmontana pandacaqui	 9	 10	2 9	 5	2 26	4	 59	.44	1.15
50. Ixora cumingii	 1	1 9	 9	 1	3 20	4	 40	.44	
51. Antidesma leptocladum		 8	1 5	 2	3	4	 10	.44	
52. Pterospermun obliquum			 1	2 2	2 6	4	 9	.44	.29
53. Aglaia bordenii	 1	 8	1 3	1 2	2 2	4	 11	.44	
54. Clausena anisum-olens			 2		4 8	4	 5	.44	
55. Casearia fuliginosa	1	 3	1 1	2		4	 4	.44	.29
56. Sterculia oblongata	1			1 2	2 1	4	 3	.44	
57. Sp. indet	2		2 3			4	 3	.44	
58. Plectronia umbellata					4 1	4	 1	.44	
59. Ficus hauili		2	2			4		.44	.86
60. Pandanus luzonensis				4		4		.44	2.02
61. Cynometra simplicifolia	2 1	 1		1 1	 4	3	 7	.33	.86
62. Semecarpus perrottetii			1 4	1 3	1	3	 7	.33	
63. Pterospermum diversifolium			 1	3 5		3	 6	.33	.29
64. Sp. indet	1		 1	1	1 2	3	 3	.33	
65. Diospyros nitida			1		2 2	3	 2	.33	
66. Illipe ramiflora			1 2	2		3	 2	.33	
67. Linociera cumingiana		 1	 1		3	3	 2	.33	
68. Artocarpus lanceolatus	1	1			1	3		.33	.29
69. Dracontomelum cumingianum	3					3		.33	1.15
70. Shorea polysperma			2	1		3		.33	
71. Litsea luzonica	 2	 5	 13	1 6	1 3	2	 29	.22	
72. Mitrephora lanotan	 5	 1	1 6	 8	1 4	2	 19	.22	
73. Dracæna angustifolia	 1	 2	 2	1 5	1 6	2	 16	.22	.86
74. Cinnamomum mercadoi					2 2	2	 2	.22	
75. Euphorbiaceæ sp	1		1		 1	2	 1	.22	
76. Buchanania florida					2	2		.22	.29
77. Crypteronia cumingii			2			2		.22	
78. Gonocaryum tarlacense	1		1			2		.22	

TABLE XIV—Continued.

Name.	A.	B.	C.	D.	E.	F.	G.	H.	I.
79. Lagerstrœmia speciosa	1			1		2		.22	4.10
80. Palaquium oleiferum			1		1	2		.22	
81. Polyscias nodosa	1		1			2		.22	.29
82. Pterocymbium tinctorium				2		2		.22	
	1						1		
83. Pygeum latifolium					2	2		.22	.29
84. Radermachera banaibana				2		2		.22	.86
85. Sp. indet			1	1		2		.22	
86. Cyclostemon microphyllum				1		1		.11	.57
	1	4	6	12	9		32		
87. Garcinia venulosa			1			1		.11	
	1	2	3	1			7		
88. Neolitsea vidalii			1			1		.11	.29
			1	5			6		
89. Sideroxylon macranthum			1			1		.11	.29
				5			5		
90. Dysoxylum cumingianum	1					1		.11	1.73
	2		1	2			5		
91. Sideroxylon duelitan	1					1		.11	
		1	2	2			5		
92. Unona clusiflora					1	1		.11	
	1		1	3			5		
93. Endiandra coriacea					1	1		.11	
	1				1		2		
94. Flacourtia inermis					1	1		.11	
					2		2		
95. Parinarium griffithianum					1	1		.11	[illegible]
				1	1		1		
96. Plectronia sp					1	1		.11	
					2		2		
97. Pahudia rhomboidea					1	1		.11	
					1		1		
98. Ixora sp				1		1		.11	
		1					1		
99. Sp. indet					1	1		.11	
					1		1		
100. Sp. indet		1				1		.11	.57
			1				1		
101. Artocarpus communis		1				1		.11	.57
102. Dipterocarpus grandiflorus			1			1		.11	
103. Ficus ampelas			1			1		.11	
104. Ficus sp			1			1		.11	
105. Ficus nota	1					1		.11	
106. Ficus variegata			1			1		.11	
107. Ganophyllum obliquum				1		1		.11	
108. Gardenia barnesii					1	1		.11	
109. Mallotus ricinoides		1				1		.11	
110. Mangifera altissima	1					1		.11	7.00
111. Planchonia spectabilis			1			1		.11	

TABLE XIV—Continued.

Name.	A.	B.	C.	D.	E.	F.	G.	H.	I.
112. Sterculia crassiramea				1		1		.11	
113. Vitex sp			1			1		.11	
114. Randia fitzalani			1			1		.11	
115. Sp. indet	1					1		.11	
116. Sp. indet	1					1		.11	
117. Sp. indet			1			1		.11	
118. Sp. indet				1		1		.11	
119. Sp. indet					1	1		.11	
120. Sp. indet					1	1		.11	
Total						896			

Storied vegetation.—An analysis of this plot, as a whole, more or less strikingly shows two stories of vegetation. In the upper one, the trees reach to the height of 25 to 36 meters. (See Pl. XII.) The species composing it are, in the main, *Dipterocarpaceæ*. Among these are *Shorea guiso* (guijo), *Shorea contorta*, (lauan) *Dipterocarpus vernicifluus* (panao), *Anisoptera vidaliana* (mayapis), *Shorea polysperma* (tanguili), and *Hopea acuminata* (dalindingin). Still others in the upper story are *Euphoria cinerea*, *Santira nitida*, *Palaquium luzoniense*, *P. tenuipetiolatum*, and *Koordersiodendron pinnatum*. In all, about 31 species, out of a total of 121 found in the plot, have representatives in the upper story.

A careful analysis of the plot will, in places at least, show that the trees of the top story are far apart. (Pl. XII.) While the crowns for a short distance are often close enough together to make an even skyline yet there are often depressions in this profile which are due to the absence of the upper-story trees. However, this irregularity is not so striking as it is in the *Bambusa-Parkia* formation. The lower-story vegetation consists of the younger trees of the upper one, together with species which seldom reach 20 meters and usually are from 10 to 15 meters in height. (Pls. XIV, XV.) Of these the most common are *Strombosia philippinensis*, *Gnetum gnemon*, *Diospyros pilosanthera*, *Aporosa symplocosifolia*, *Reinwardtiodendron merrillii*, and others.

Relation to topography.—The table shows differences in vegetation corresponding to types of topography. In Plots A and E the gradient is near 40 degrees, and corresponding to this, there are fewer tall trees and many more saplings and seedlings. E is on the brow of the hill, and much more exposed to winds, the soil is shallower and stony, and no doubt the chresard is much lower. Less mesophytic species such as

Atalantia disticha, Memecylon edule, Dimorphocalyx longipes, Xylopia dehiscens, Tabernæmontana pandacaqui, and *Ixora cumingii* are found at the extreme upper limits of the plot and with others in the adjacent territory very much resemble the low parang vegetation. Indeed, the entire brow of the hill is covered with the tangled growth of the climbing bamboo, *Dinochloa diffusa.* Of the mesophytic species *Strombosia philippinensis* and *Gnetum gnemon* are representative. While they are prominent in the subplot E, yet they occupy the lower half of it. (See Pl. XV.) As a rule the *Dipterocarpaceæ* are found to be best developed in Plots C. and D. Here the slope is more gentle.

A comparison of the entire hillside with the river terrace shows differences other than those which have been mentioned. *Anisoptera vidaliana,* occupying first place in the river terrace, stands eleventh on the hillside. *Palaquium luzoniense* is very common below, but it is represented only by a few scattered specimens above. A reference to columns H and I of Table XIV and B and C of Table XIII will strikingly show these differences. To give a better idea of the complexity of the floristic composition of the forest and of the differences due to physiographic situations, six other plots were made in this *Anisoptera-Strombosia* formation.

Description of Table XV.

This table classifies plots in various physiographic situations of the *Anisoptera-Strombosia* formation. A, forest on a slope of 10 to 12° gradient at an altitude of 100 meters. The plot comprises an area of 1500 square meters. B, forest on a terraced island at an altitude of 80 meters. This terrace is under water for a very short time at extremely high floods. The plot comprises an area of 600 square meters. C, plot in the river terrace a little higher than B. It is near the mouth of a wet-weather stream and is flooded at the highest water. It is near Plot A and comprises about 800 square meters. D, plot on a terrace about 2 meters higher than B. The plot comprises an area of 1,400 meters. E, plot on a level bench at 250 meters altitude. It comprises an area of about 800 square meters. F, plot on a gentle slope at the top of a ridge at 190 meters altitude. It comprises an area of 750 square meters.

TABLE XV.

Name.	A.	B.	C.	D.	E.	F.	G.
1. Aphananthe philippinensis	-------	5	27	27	-------	-------	59
2. Anisoptera vidaliana	9	8	13	6	8	4	48
3. Lagerstrœmia speciosa	-------	7	13	19	-------	-------	39
4. Canarium villosum	-------	3	9	14	-------	-------	26
5. Memecylon edule	-------	4	7	11	1	1	24
6. Dipterocarpus vernicifluus	1	5	-------	4	2	7	19
7. Artocarpus communis	-------	3	3	9	-------	1	16
8. Koordersiodendron pinnatum	3	4	3	1	3	1	15
9. Shorea polysperma	-------	-------	-------	-------	10	5	15
10. Shorea contorta	-------	-------	-------	-------	10	4	14
11. Grewia stylocarpa	3	-------	-------	-------	4	6	13

TABLE XV—Continued.

Name.	A.	B.	C.	D.	E.	F.	G.
12. Buchanania florida			2	8		1	11
13. Champereia cumingiana	1		3	7			11
14. Baccaurea tetrandra	2				5	3	10
15. Calophyllum wallichianum	1				5	2	8
16. Knema heterophylla	8						8
17. Semecarpus perrottetii		1	2	4		1	8
18. Strombosia philippinensis	6					2	8
19. Diospyros pilosanthera					2	5	7
20. Gnetum gnenom						7	7
21. Streblus asper		1	3	3			7
22. Adinandra pavonina		1		5			6
23. Chisocheton philippinum	5					1	6
24. Mallotus philippinensis				6			6
25. Parkia roxburghii	1	2	1	1		1	6
26. Santiria nitida	2				3	1	6
27. Shorea guiso	1			3	1	1	6
28. Ficus hauili		1	3	1			5
29. Pithecolobium acle	1	1	1	1		1	5
30. Pterocymbium tinctorium	4			1			5
31. Talauma villariana	1	1			2	1	5
32. Aporosa symplocosifolia		1			2	1	4
33. Cryptocarya luzoniensis	1				2	1	4
34. Palaquium tenuipetiolatum					2	2	4
35. Terminalia edulis		2		2			4
36. Aglaia turczaninowii				3			3
37. Canarium ahernianum					2	1	3
38. Dracontomelum cumingianum	1				2		3
39. Endiandra coriacea	1				2		3
40. Erythrina indica		3					3
41. Eugenia luzonensis		3					3
42. Euphoria cinerea		1			1	1	3
43. Garcinia venulosa		1	1	1			3
44. Grewia tiliæfolia				3			3
45. Hopea acuminata						3	3
46. Myristica philippensis					2	1	3
47. Palaquium oleiferum				2		1	3
48. Parinarium griffithianum		1				2	3
49. Symplocos polyandra		1			2		3
50. Turpinia pomifera	2					1	3
51. Xanthophyllum sp	2					1	3
52. Aglaia denticulata	1					1	2
53. Artocarpus lanceolata						2	2
54. Canangium odoratum		2					2
55. Carallia integerrima	1				1		2
56. Casearia fuliginosa				2			2
57. Cinnamomun mercadoi						2	2
58. Cyclostemon bordenii	1					1	2
59. Macaranga bicolor		2					2
60. Mangifera altissima			1	1			2
61. Mitrephora lanotan	1					1	2
62. Linociera cumingiana				2			2
63. Pittosporum pentandrum				2			2
64. Planchonia spectabilis		1			1		2
65. Unona clusiflora	1			1			2
66. Pterospermun obliquum			1			1	2

TABLE XV—Continued.

Name.	A.	B.	C.	D.	E.	F.	G.
67. Wrightia laniti			1	1			2
68. Zizyphus trinervis			1	1			2
69. Zizyphus zonulatus				2			2
70. Aglaia harmsiana			1				1
71. Allæanthus luzonicus	1						1
72. Antidesma leptocladum						1	1
73. Dysoxylum cumingianum	1						1
74. Arytera littoralis		1					1
75. Celtis philippinensis						1	1
76. Cyclostemon monospermus	1						1
77. Cyathocalyx globosus		1					1
78. Cyclostemon microphyllum						1	1
79. Garcinia binucao				1			1
80. Ellipanthus luzoniensis	1						1
81. Endospermum peltatum	1						1
82. Eugenia bordenii						1	1
83. Ficus nota						1	1
84. Flacourtia inermis	1						1
85. Glochidion sp				1			1
86. Gonocaryum tarlacense						1	1
87. Harpullia arborea			1				1
88. Laportea luzonensis		1					1
89. Litsea perrottetii		1					1
90. Homalanthus populneus				1			1
91. Macaranga mappa		1					1
92. Memecylon preslianum					1		1
93. Palaquium luzoniense			1				1
94. Premna nauseosa				1			1
95. Premna cumingiana		1					1
96. Quercus sp						1	1
97. Reinwardtiodendron merrillii	1						1
98. Sterculia oblongata	1						1
99. Tarrietia sylvatica		1					1
Total							559

In the above plots, A, B, C, and D are on the border of the *Anisoptera-Strombosia* and the *Bambusa-Parkia* formation; consequently the vegetation partakes of the nature of both. The ones on the low river terraces show a strikingly different floristic and ecological composition from those on the slopes and more closely resemble the river terrace plot at an altitude of 100 meters. (See Table XIII.) Such species as *Aphananthe philippinensis*, *Lagerstrœmia speciosa*, *Canarium villosum*, and *Memecylon edule* are very suggestive of *Bambusa-Parkia* formation, and indeed these areas might be classed with the latter were it not for the absence of the bamboo. It is not at all unlikely that the physical factors more nearly resemble the *Bambusa-Parkia* than the *Anisoptera-Strombosia* formation. Only a careful measurement would determine this.

On the other hand, Plots E and F are nearer the *Dipterocarpus-Shorea* formation and consequently have some resemblance to it, both floristically

and ecologically, as is shown by the occurrence of such species as *Shorea polysperma, Shorea contorta, Calophyllum wallichianum,* and *Hopea acuminata* (compare Plots E and F, Table XV, with Table XVI). *Anisoptera vidaliana* is fairly well distributed throughout all the plots, and for this reason the formation shares its name with *Strombosia.*

Lianas.—The liana vegetation of the plot on the river terrace at 100 meters altitude has already been discussed. Apparently its development here is much greater than it is in the closed forests, but in reality the difference is not very pronounced, though there is no doubt that the lianas occupy proportionately more space in the open than in the closed forests. (Pl. XIV.) Though the shade of the forest practically prohibits most lianas from obtaining a start from seed, yet they, as has been previously shown (p. 408), are not entirely dependent on seeds for the invasion of new territory. Especially is this true of the climbing bamboo and of at least one species of *Calamus,* though dicotyledinous lianas may also have this power. Even though the lianas did not possess this property, natural and artificial "blanks" occur in the forests and these would give opportunity for lianas together with other species to obtain a foothold from seeding.

Rattans.—Contrasted with the undergrowth of temperate regions, the species of the genus *Calamus* are most foreign. In their younger stages these climbing palms consist of a ground rosette of large leaves having their leaflets pinnate or arranged in groups, and, either above or below or on both the upper and under side, containing scattered, thin, flexible spines on the principal veins. In many cases the rachis of the leaf is prolonged into a long, whip-like runner (flagellum) having stout recurved hooks, the latter often arranged in groups; the leaf sheath is also strongly armed and may itself be provided with a long flagellum which in turn is armed. In time, from the center of the basal rosette, there arises a long scandent stem, the rattan of commerce, which may reach to the tops of the tallest trees or it may scramble over the ground, sometimes reaching a total length of 120 meters or more. After these stems are well developed, the basal rosette disappears. Among the species common at Lamao are *Calamus mollis major* (Uay), *C. ornatus philippinensis* (Limoran), and the undetermined species having the native names of *Dilan, Palasan, Culacling, Babuyan,* and *Bucton.* (Pl. XV.) Of these, *Bucton* has a distinct, though short, trunk so that the basal rosette stands at least 20 centimeters above the ground. *Limoran* has the habit of producing runners and of thus starting new centers at some distance from the mother plant. These, in turn, may throw out runners which enable the plant to spread without going through the dangerous seedling stages. Without exception, all the species are able to endure the most intense shade, although they, like other lianas, present a much better display of foliage, at least nearer the ground, when they grow in half open places. (See Pl. XVI.) They also share with the climbing

bamboo and other forms, the habit of creeping into "blanks" in the forest and they thus retard what would otherwise be the rapid entrance of tree species into such places. Usually, the rattan growth is scattered through the forest (see Pl. XV), but often individuals are so densely set (Pl. XVI) that it is difficult to pass through such a location because of the armed flagella which, curving downward, catch the clothing. By occupying space and by increasing the already dense shade, they often prevent the germination and development of valuable tree species. With the exception of *Uay,* all the species of *Calamus* here are mesophytic.

Other lianas.—Other families represented by lianas are the *Pandanaceæ* and the *Araceæ.* Characteristic of the former is the genus *Freycinetia* with the species, *F. luzonensis* and *F. ensifolia. F. luzonensis* climbs to the tops of largest trees, the ends of its shoots bend stiffly outward and upward and develop closely crowded leaves which give a characteristic "pandan" tone to the vegetation. Often species of *Araceæ* of the genus *Pothoidium* and *Rhaphidophora* begin their growth semi-epiphytically near the bases of large trees and afterwards climb by means of roots; at the same time, positively geotropic roots reach to the soil and insure a more constant supply of water to the older plant.

Dicotyledinous forms present on the river plain are also encountered in the forest. Besides the ones already mentioned, *Artabotrys cumingianus, Agelæa wallichii, Strongylodon macrobotrys, Entada scandens, Modecca sp., Uvaria sp.,* and others (see Pl. XIV) may also be present.

Transition from epiphytes to lianas.—The transition from the epiphyte to the liana is best expressed by the genus *Ficus* of the section *Urostigma.* (See Pl. XVII.) The species of this group begin as epiphytes on the rough surface of the bark or on other parts of the tree where the seeds can find lodgment; here they germinate, the roots eventually reaching and penetrating the ground. In time there is developed a lattice-like growth which envelopes the host, and then, as the parts increase in diameter, the plant assumes a tree-like habit which enables it to exist independently of its host, which eventually dies. The natives term the species of *Ficus* which have developed this habit "balete," which, to some extent, species of *Schefflera* of the *Araliaceæ* simulate. Plate XVIII shows a seedling of *S. venulosa* which has developed on soil which collected in a knot hole of *Pithecolobium acle.* Plates XVII, XVIII, XIX, and XX depict species of *Ficus* in several stages of maturity. Often from branches of the plant there are developed one or more large roots which eventually reach the ground. From the base to the top of the mountain species of "balete" are found scattered throughout the forest.

Epiphytes.—The epiphytic vegetation in contrast with that of the higher altitudes is but poorly developed. Mention has been-made already of the orchids of the river terrace. The rough limbs of *Terminalia edulis,* which grow in more or less protected places, seem to be a favored

position for many individuals of *Cymbidium aloifolium*, *Aerides quinquevulnera*, and *Rhynchostylis violacea;* on the higher and more xerophytic parts of the tree there were many specimens of the fern *Drynaria quercifolia.* The branches of *Lagerstrœmia speciosa, Ficus caulocarpa,* and other trees afford a good surface for the germination of orchid seeds; the semiparasitic *Hoya multiflora* is also not infrequently encountered. Several species of orchids, and the ferns *Drynaria, Vittaria lineata,* were noted on the branches of the tall *Dipterocarpaceæ.* Species of *Loranthaceæ*, although present, are less common than they are in the parang.

Herbaceous vegetation.—One of the surprises of the forest is the scarcity of herbaceous plants growing in the dense shade during both the dry and wet seasons. Even in the open places there are found only a few geophilous *Araceæ*, some grasses, small species of *Acanthaceæ* as *Gymnostachyum affine, Hypoestis subcapitatus, Lepidagathus hyalina,* and others. Scattered throughout the forest is the sedge *Hypolytrum compactum.* This species, with a few ferns and *Begonia rhombicarpa* on the more damp slopes, are the only truly herbaceous plants which were noted in the dense forest.

Plank buttresses.—Schimper[37] has called attention to the prevalence in tropical countries of trees with plank buttresses or board roots. He claims that these are a peculiarity in a tropical climate where the rainfall is abundant and that they are found in the evergreen-rain, and in most of the deciduous, monsoon forests.

Prevalence of buttresses.—In the *Anisoptera-Strombosia* formation out of 39 fairly mature trees 15, or 38 per cent, had no buttresses, 13, or 33 per cent, had buttresses over 0.5 meter, and 11, or 29 per cent, less than 0.5 meter in height. The buttresses assume various shapes. Some extend up the trees to a height of 3 meters or more and are less than a meter in width; others are as broad as they are long; still others, usually low ones, branch. Of those observed, species of *Anacardaceæ* (*Dracontomelum cumingianum* and *D. mangiferum*) exhibit the best-developed ones, the buttresses of one specimen of *D. cumingianum* being 3½ meters in height by 3 in width. As a rule, young trees show but little or none of this development. Old trees of *Dipterocarpus vernicifluus* may or may not have them. One of these showed a buttress which was narrow but at least 7 meters in height; another equally large tree had none. *Eugenia glaucicalyx* has three equally strong buttresses which are developed in such a way as to divide the circumference of the trunk into three nearly equal parts. (See Pls. X, XI, XII, XXI, XXII, XXIII.)

Cause of buttresses.—The buttresses seem to be correlated with broadly developed crowns (Pl. XII). *Dracontomelum cumingianum*, for instance, a tree usually growing in open places, has long, scraggy branches with coarse twigs on the ends of which are rosettes of large, compound leaves. In the forest where the crowns are dominant, and where they

[37] Loc. cit., 304, 305.

have an opportunity to develop horizontally as well as vertically, the buttresses are correspondingly large. This horizontal growth is especially marked in the *Bambusia-Parkia* formation, where the dicotyledinous trees overtop the bamboo. Here *Parkia* itself shows strong buttresses. In the formation higher up the mountain the buttress habit is present, but not so prevalent; it disappears altogether on the exposed ridges of the mountain.

It will be seen that the same causes which operate to produce short, thick boles in open places in temperate regions are possibly also present here, with the difference that the buttresses, which take the place and perform the function of the uniformly thick trunks, appear later in the life of the tree. This means that the tree in youth, while it is crowded in the forest, develops a regular bole, but that when it reaches above the surrounding vegetation, in response to the increasing heaviness of the top, the buttresses appear at the base, enabling the tree to withstand the extra strain. It is possible that this of itself is the stimulus causing the development. It is known that the presence of heavy limbs often produces extra growth in the trunk at a point just beneath the insertion of the branches, and the fluted appearance so commonly observed in such cases is the result. The cause for this growth is ascribed to the stimulus due to the extra strain, although why such a stimulus should produce this result is not known. A transference of the strain to the base of the trunk would likewise cause prolific growth at the point or points where its influence is felt the strongest; this point would be at the attachment of the roots, and especially of the larger ones, and thus would cause the development of board roots or plank buttresses. So long as the tree top is protected on all sides by others, the strain will not be very great for two reasons, i. e., the top will not have a chance to broaden out and it will not be exposed to strong winds, but so soon as it emerges above the tops of other trees, the exposure and the increasing heaviness above will produce a strain sufficiently great to cause a stimulus of growth at the base and thus produce the buttresses. However, whatever may be the cause, there is no doubt that the abundance and size of buttresses in many instances is associated with the *dominance* of certain trees over others, and thus the irregular profile of the forest results. This is strongly emphasized by the fact that in places where the trees reach a more uniform diameter and height and where they are set sufficiently close to produce a class with an *intermediate* crown, or, to express it in another way, to form a regular profile, the buttress habit is not so pronounced. Such is the case in places in the *Anisoptera-Strombosia* formation and it is more prevalent in the *Dipterocarpus-Shorea* one which is to be discussed below.

Trunks.—The shape of the bole of the tree is of the greatest importance to the forester. Other things being equal, the more nearly the bole reaches a true cylinder throughout its entire length, the more lumber will it produce. In other words, the more nearly the *form factor*

reaches *one* the higher the value of the log. It has been shown that for some species of trees the board roots are not developed so long as the trees do not become *dominant*. If it be desired to suppress the buttress habit for such species, it will be found effective to adopt a sylvicultural treatment which will keep species of the same *crown class* together, so that throughout the entire rotation none will become dominant. In some parts of the Lamao Reserve such a system is already naturally realized. On the other hand, it may be expedient as fully as possible to develop the board roots, for certain timbers are valuable for broad table tops. In such cases a sylvicultural system, the reverse of the one just given, would produce the desired results. If the strain is the cause of the development of plank buttresses, then this could be concentrated by the removal of the less desirable ones, so that the others would receive more of it and thus become stimulated to better development.

Fluted trunks.—In some trees at the top and at the base of the bole there is a tendency to develop a fluted appearance. In such individuals, especially if the trunk is short, the grooves are apt to be more or less continuous throughout the entire length, although the middle portion is more nearly circular in cross section. *Euphoria cinerea, Eugenia luzonensis* (Pl. VI), *Dracontomelum cumingianum, Reinwardtiodendron merrillii, Turpinia pomifera,* species of *Ficus,* and others may have such irregular trunks. Many trees do not have the buttress habit in any situation. Among those which may sometimes become dominant are species of *Anonaceæ*, as *Cyathocalyx globosus* and *Canangium odoratum,* and in other families *Bombax ceiba, Zizyphus zonulatus,* and of those which seldom reach above the second-story vegetation, *Strombosia philippinensis, Antidesma edule,* and many others may be mentioned.

Tree habit.—In the *Anisoptera-Strombosia* formation, aside from *Bambusa, Gnetum gnemon,* and *Pandanus luzonensis,* there are only a few scattered individuals of plants that have acquired the tree habit in the great groups of *Pteridophytes, Gymnosperms,* and *Monocotyledons.* *Gnetum gnemon,* as shown by Tables XIII and XIV, forms a conspicuous element in the second-story vegetation of the forests. It rarely reaches a height of more than 12 meters. It is easily distinguished by its very broad, opposite mesophytic leaves and the joints of the trunk. These latter cause the bole to resemble a bamboo with short, thick internodes. (See Pl. XIV.) The liliaceous *Dracæna angustifolia,* represented only by a few trees over 4 meters tall, is nevertheless conspicuous by many specimens under this height and by its long, narrow, clasping leaves. Only one specimen of a tree fern, *Alsophila contaminans,* is present (see Pl. XXVI). This will be discussed in another connection. Near the upper limits of the *Bambusa-Anisoptera* formation there are two groves of the palm *Orania palindan,* and a few scattered specimens were noted elsewhere. One grove of eight trees of *Cycas circinalis* was found in a semi-open place within this formation.

Shape of crown.—Aside from the regular oval or irregular tree crowns, a number of types worthy of mention were noted. ***Polysias nodosa,*** which in the parang usually has a single rosette of large compound leaves, in the closed forest reaches tree dimensions of considerable size and has from three to six short branches at the top, each of which ends in a rosette of leaves. Many ***Meliaceæ*** have similar crowns; in young trees there is a single rosette, and in older ones there are rosettes on the ends of secondary and tertiary branches. This, as already shown, is the habit of ***Dracontomelum cumingianum. Koordersiodendron pinnatum*** has a similar habit. ***Orania palindan, Cycas circinalis,*** and a tree fern, ***Alsophila contaminans,*** are among the smaller trees which have a single rosette of compound leaves. Of those which have a single rosette of large simple leaves are ***Semecarpus gigantifolia, Macaranga mappa,*** and ***Ficus psudopalma.*** By far a majority of trees have an oval or spherical crown or a more or less irregular one. Aside from compound leaves of members of the ***Leguminosæ,*** the ***Meliaceæ, Burseraceæ,*** and ***Anacardiaceæ,*** most of the trees have simple, entire leaves. Those with a serrate or wavy margin are the exception. Guttered leaves are represented, though sometimes obscurely so, by species in the ***Moraceæ (Ficus), Guttiferæ (Calophyllum*** and ***Garcinea), Myrtaceæ (Eugenia),*** and others.

Bark of trees.—Nearly all writers on tropical vegetation have called attention to the thin bark of tropical trees as compared with those growing in temperate climates. This is due to the weak development of the corky tissue. The measurement of the bark of 44 mature or nearly mature trees, representing different species, shows an average thickness of 6.87 millimeters. Of those measured, ***Palaquium luzoniense,*** with a bark 15 millimeters in thickness, represents the maximum, and ***Antidesma edule,*** with a bark 2 millimeters thick, the minimum. The cause of the weak development of the corky tissue is generally attributed to the effect of humidity on the formation of bark. Where the humidity differs, observations have not been extensive enough to show any appreciable variation between the development of bark on the same species in the trees of the Lamao Reserve, found in two or more plant formations. (See Pls. X, XI, XIX, XXI, XXII, XXIII, XXIV, and XXV, for bark characters.)

Notes collected in reference to the bark of nearly a hundred different species show that in a majority of cases this is smooth, and light gray or brown in color. In many instances the surface is more or less thickly set with *corky pustules* of various shapes and sizes. In the case of the rhamnaceous ***Zizyphus zonulatus,*** short, thick pyramidal thorns are characteristic. In ***Diospyros pilosanthera*** the pustules are more or less thorn-like, but do not assume any regular shape. In many trees with more or less smooth bark, it is shed at irregular intervals in large or small scroll-like patterns. In the myrtaceous ***Tristania decorticata,*** as the specific

name implies, the whole bark is shed periodically. In this respect it resembles the closely related *Eucalypti*. In other genera of *Myrtaceæ* the bark becomes papery. In *Eugenia luzonensis* the corky layer, which is a light red, peals off in this papery fold very much like the birch. In *Eugenia bordenii* the thin layers are light gray instead of red. While gray or brown are the predominating colors of barks, yet there are exceptions. In *Diospyros pilosanthera* and other species it is nearly black. A yellow bark distinguishes *Calophyllum wallichianium* at once. In many cases the bark is ridged. Usually the ridges are low and correspondingly the depressions are shallow. Of course, in many species the rough barks do not appear until the tree has attained some degree of maturity. Among these trees which have rough barks may be mentioned *Palaquium luzoniense, Anisoptera vidaliana* (in very old age), *Shorea contorta, Koordersiodendron pinnatum,* and others. In *Pithecolobium acle* and in *Bischofia trifoliata* the corky layer consists of choppy flakes turning outward at the free, lower edge.

Sap of bark.—Aside from the outer, the middle and inner barks often have distinctive characteristics which enables one to place them in the family. In the case of the *Anonaceæ* the strongly marked, medullary rays of the inner bark form alternating bands parallel with the radii of the trunk. The exudation of sap from the cut bark also is often striking, and with or without other characteristics will enable one to identify the families. Thus, in the *Myristicaceæ* represented by *Myristica* and *Knema,* the bark when cut freely exudes a red sap. In the genera *Calophyllum* and *Garcinia* of the *Guttiferæ* the sap is yellow. In the *Dipterocarpaceæ* a nearly colorless resin is slowly exuded. In the *Burseraceæ,* in species of *Canarium* and in *Santiria nitida,* a similar resin is found which burns readily. The white and often abundant milky sap of the trees in members of the *Moraceæ, Apocynaceæ,* and *Sapotaceæ* will distinguish these families from others, though often other characters are necessary to tell them one from the other.

It will be seen from the above that frequently there are family, generic, or specific differences substantial enough to warrant the collecting of data for a key which will enable one to distinguish the many species by bark characteristics. Undoubtedly, the need of such a key for the practical forester is great, for the difficulty of obtaining flowers or even leaves from the tall trees, coupled with the multiplicity of species, makes the task of mere determination a difficult one.

Cauliflory.—Schimper [38] associated cauliflory with thin bark characters. Other authors associate it with conditions of pollination. Buscalioni [39] claims that the cauliflower habit is a primitive one and persists in plants of hot, moist regions. The ecological advantages are numerous. In

[38] L. c. 336.

[39] Buscalioni, L., *Sulla cauliforia. Malpighia* (1904) **18:** 116–176. A full bibliography of the subject is given with this paper.

many cases heavy fruits are produced which, if not attached to a strong, woody stem, would be easily broken off. Such is the case in many *Meliaceæ*, where the fruits often become large, and, when grouped on one stem, very heavy. *Ficus minahassæ* (see Pl. XXVI) has a fruit stalk which often is 3 meters in length and thickly covered with fruit. No small twig could possibly support such a weight. An enumeration of the plants of the Lamao Reserve which possess cauliflory would include species in many of the large families. However, instances where the trunk bears the flowers or fruit are not common. Among those observed were *Eugenia whitfordii, Ixora macrophylla,* and a number of species of *Ficus.* An undetermined species of the anonaceous *Goniothalamus* had the short flower-stalk springing from a root at the base of tree. Very numerous are the trees which exhibit an attachment of the flowers to the smaller and larger branches. Another significance ascribed to cauliflory is protection of both the flower and the fruit from too much heat and rain.

Clearings in forest.—Clearings made in the midst of the *Anisoptera-Strombosia* formation often by Negritos, usually fill up rapidly with climbing bamboo and are similar in character to those described under the *Dinochloa* parang. However, no extensive tracts have ever been cleared. In Plate X is shown a river terrace from which the second-story growth has been taken. Plate XXVII is a view of a portion of this same terrace about two years later. The forest was opened sufficiently so that light-loving species could thrive. Many weeds (see p. 395) had gained a foothold and other species enumerated in connection with the plate have invaded this region from an open, rocky terrace, near by. That the clearing will quickly recover its normal condition is evidenced by the fact that species of the standing trees already have seedlings in the new growth.

Seedlings.—As most of the data for this paper were collected during the dry season, there has been little opportunity to study the early condition of the seedling process. The tables in which are given the actual number of young trees under 4 meters in height show some results. For instance, *Anisoptera vidaliana* in the open places on the rocky terrace (Table XIII) show many more young than old trees, while on the more closed adjoining hillside (Table XIV) there are fewer young than old ones. In *Parkia roxburghii* the difference is still more striking. The conclusion is that these two trees are exceedingly intolerant of shade. This is especially true of *Parkia.* After the rainy season sets in, many seedlings of *Anisoptera* were observed in rather shady forests, though they were always more plentiful in open places. On the other hand, *Dipterocarpus verniciflùus* shows a large number of young trees in the closed forests, and corresponding with a less number of larger trees in the open places, there are fewer seedlings. During the rainy season many seedlings of this tree were found in very dense shade. The large, winged seeds germinate lying on top of the ground. In open places, even well stocked

with seeds, only a few seedlings were found. This indicates conclusively that the shaded conditions are the best for this species. Facts deduced from the table and from some observation during the rainy season show that *Koordersiodendron pinnatum* resembles *Anisoptera* in its seeding habits; that is, it will tolerate shade, but it does better in more open places. Correlated with the light-loving habit one finds the seed of *Koordersiodendron pinnatum* and *Parkia roxburghii* germinating to some extent before the rainy season sets in. On the other hand, *Anisoptera* did not, though many seeds were present. *K. pinnatum* has a fleshy fruit which is eaten by the wild hogs. The seeds, however, pass through the intestines of the animals undigested and are buried in loose ground made by the rooting of the hogs, where in a few days they germinate. Thus the seeds may not only be carried long distances by hogs, but are also planted by them. However, the young tender seedlings which germinate may be eaten by the same animal, so that the advantages which this tree would have over others is partially counteracted by the same agent. Many very young trees of *Calophyllum wallichianum* were observed both in open and closed places, though in the latter they are most numerous. On the other hand, many species represented by larger trees show no seedlings at all. *Lagerstrœmia speciosa* is a striking example of this. Though seeding abundantly during both seasons in which it was observed, not a single seedling could be found. Efforts to germinate these seeds in the nursery proved unavailing. Species of *Dipterocarpaceæ* other than those discussed show an average number of young trees present, but as these species did not fruit abundantly during the two seasons under consideration, the sylvicultural habits as regards light can not with certainty be determined. A reference to the tables will show the proportion of young to old trees in other species. Much work remains to be done on the seedling condition of the principal forest trees before any definite conclusions can be reached.

Topography and soil.—The topography of the *Anisoptera-Strombosia* formation consists of a series of ridges alternating with deep cañon-like valleys. The gradient of the slopes of the valleys is approximately 25 degrees, although, of course, it may be more or less. We have seen that corresponding to the surface diversity there are types of forests which differ in species and in leaf structure. Here, as in other formations where the slope is steepest, the surface wash is greater and consequently the soil is shallow. Usually, also, the exposure is great. All these combine to make a lower chresard and consequently a more xerophytic vegetation.

Especially are these conditions obtained on the ends of ridges which terminate abruptly at the junction of the branches with the main streams of the river. Attention has also been called to the character of the vegetation on the rocky flood plains of the river. Here, though the underground water level is rather near the surface yet, because of the nearly absent soil and the hydrodynamic effect of the flooded stream, the vegetation is inclined to be more or less unstable and consequently open.

Slightly higher terraces where the surface wash of the slopes adjoining them has been deposited, exhibit the best conditions for growth. However, such terraces are narrow. On the gentle slopes, with a gradient of 20° and under and on top of the level ridges, good vegetation conditions are obtained.

The soil, as already shown, is composed of a heavy clay, the residual of the andesitic rocks of the mountain. The depth, even on rather steep slopes, is surprisingly great. All through the formation, holes in many instances 2 meters deep made by Negritos in search of the wild yam occur. In some instances the residual soil on narrow level ridges is as much as 3 meters in depth and perhaps more. In many places, however, the disintegrated portion of the rock is at or near the surface; in such places the vegetation is thin. Here deciduous or evergreen trees with xerophytic structures are common.

An analysis [40] of soils collected on the tops of ridges and on terraces shows the following:

TABLE XVI.

	H_2O, 100°-105°.	Loss on ignition.	CaO.	P_2O_5.	N.	K_2O.	Na_2O.	Humus.	Water capacity.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
I	6.98	11.89	0.12	0.06	0.03	0.11	0.23	3.99	54.06
II	7.59	13.36	.26	.08	.12	.09	.20	2.77	56.71
III	6.99	8.45	1.05	.07	.15	.12	.98	2.28	51.92
IV	6.34	11.29	.22	.13	.13	.09	.51	3.29	51.30

Description of soils.—I is the surface soil of a ridge; II, collected at depth of 15 centimeters in the same situation; III and IV are surface soils collected from terraces.

Humus.—It will be seen that the soils are not deficient in essential elements and that the water-holding capacity is high. The absence of humus in the soil of tropical forests has long been a subject for discussion. Schimper,[41] quoting Wohltmann, claims that humus covers smaller areas in the Tropics than in temperate zones. In South India, however, about one-third the area is covered with a rich soil containing 8 to 9 per cent of organic matter. The analyses of the soils in question show an average humus content of 3.09 per cent, which in comparison with twenty soils [42] given by Stockbridge is rather high. Of the twenty which he mentions, the Lamao soils are surpassed by only three. Nevertheless, in comparison

[40] All soil analyses given in this paper were made by L. A. Salinger of this Bureau. The methods for chemical analysis are those adopted by the Association of Official Agricultural Chemists. See Bull. 46 (revised edition), *U. S. Dept. of Ag. Div. of Chem.* Water capacity, loss on ignition, humus, and loss of water at 100°-105° are calculated on air-dry soil.

[41] L. c., 380.

[42] Stockbridge, H. E. Rocks and Soils, 250, 1895.

with the mass of half-decayed organic material of the beech or maple forests which is so prominent in some places in temperate regions, the Lamao soil is poor. The intermediate stage between the forest litter and the almost completely disintegrated organic matter is passed over quickly in the Tropics. This, perhaps, will account for the impression that the humus accumulation is not great. It must be remembered that the forces which bring about the decomposition of complex organic compounds, both of plants and animals, are much stronger in the Tropics, and because of the continuous warmth they are continually at work. Thus there is no accumulation of organic, decaying matter in the intermediate stages. In temperate regions, on the other hand, the accumulation during the whole year is greater than can be disposed of during the short, warm period in which nitrifying bacteria and other agents of decay would be operative; consequently, each year a surplus is added to the constantly increasing mass. In regions of great rainfall, where during heavy rainstorms the surface becomes a sheet of water, a great deal of organic litter is washed into the streams and thus lost to the forest. Especially is this the case in regions of rough topography, where the drainage is excellent.

Correlated with the scarcity of half-decomposed organic matter is a rather scanty development of fleshy fungi, though at the beginning of the rainy season a number of forms appear. Woody fungi, however, are constantly in evidence, usually on old logs or standing dead trunks. Among the genera present may be mentioned *Auricularia*, *Schizophyllum*, *Sterum*, *Polystichus*, *Polyporus*, *Fomes*, and *Trametes*. Mosses and liverworts are scantily represented. Lichens are found abundantly on the smooth bark of the trees and on boulders. Other than the crustaceous forms are almost entirely absent.

Many of the ecological and structural characteristics of the *Anisoptera-Strombosia* formation are also present in the other formation, so that a repetition of this matter will not be necessary. However, where differences occur, attention will be called to them. It has been noted that in the *Bambusa-Parkia* formation many xerophytic and tropophytic structures appear. In the *Anisoptera-Strombosia* formation the same condition is obtained, but to a less extent. The evergreen element is much more pronounced and the deciduous nature correspondingly less characteristic. This is in accord with the more mesophytic conditions which prevail here in contrast with the lower formation. However, as shown by the tables, many plants prevail in both habitats. Trees like *Parkia*, *Lagerstrœmia*, and *Ficus* with a partial deciduous habit are present, though less conspicuous in the *Anisoptera-Strombosia* than in the *Bambusa-Parkia* formation.

Summary.—1. In the *Anisoptera-Strombosia* formation a two-story vegetation prevails; the bamboo story of the *Bambusa-Parkia* forest is replaced by small dicotyledinous trees represented by *Strombosia*.

2. *The forest conditions and consequently forests are less xerophytic and tropophytic during the dry season; though the deciduous habit is present, no trees are deciduous throughout the entire dry season. This brings the forest more nearly in accord with Schimper's tropical rainy forest than the Bambusa-Parkia formation.*

3. The two-story vegetation gives prominence to certain dominant species, among which members of the *Dipterocarpaceæ* predominate both in number and actual volume.

4. The development of plank-buttresses is often associated with the dominant trees.

5. The great floristic complexity of the forest is in striking contrast with that of temperate forests.

6. Within the formation, various physiographic situations support different types of vegetation. The mesophytic slopes and narrow terraces support the climax vegetation, the rocky, flooded terraces are unstable, and the exposed ridges a more or less xerophytic vegetation.

[TO BE CONTINUED.]

ILLUSTRATIONS.

PLATE I. View looking southward from Buenavista Peak to Cabcaben Peak along the rim of the crater, which is on the right. The tree with an umbrella-shaped top seen on Cabcaben Peak, the ridge leading to it, and on the rim of the crater is *Leptospermum amboinense.* The rock in the foreground is in Plot H, Table XXIII. *Freycinetia* sp., a liana, is in front of it. Behind the rock on the right is *Eugenia congesta,* on the left are *Photina luzonensis* and one specimen of *Vernonia arborea.*

II. Looking across the cañon near the head of the Lamao River to Caybubu Peak and the ridge leading to it. Caybubu Peak is shown on the left. The steep slopes of the cañon are densely wooded except where almost perpendicular sides have made conditions too unstable for tree vegetation.

III. View of parang vegetation. The large tree is *Parkia roxburghii* covered with a species of *Loranthus. Parkia* is in the process of forming new leaves. Note the jungle-like thicket beneath. *Bambusa-Parkia* formation.

IV. View in the "parang." In the center is *Artocarpus communis.* Below it on left, the small tree with a rosette of large leaves is *Ficus pseudopalma. Ficus hauili, Leea manillensis, Tabernæmontana pandacaqui,* and *Gmelina hystrix* are also present. Note the abundance of liana species. *Bambusa-Parkia* formation.

V. View on the edge of a clearing. On the left is *Areca catechu;* on the right a coppice growth of *Artocarpus communis.* In the background a dense growth of the exotic species *Prosopis juliflora,* which is common near the coast. *Bambusa-Parkia* formation.

VI. View in an easily flooded, dry river channel. On the right and left are specimens of *Eugenia luzonensis;* in the center is a large specimen of *Pandanus luzonensis.* Beneath is dense growth of the marantaceous *Donax arundastrum.* The flood plain here is sandy. *Anisoptera-Strombosa* formation.

VII. View on river terrace about 3 meters above low water. In the center is a specimen of *Pandanus whitfordii;* on the left of it is *Garcinia venulosa* with *Dinochloa diffusa* over it. In the foreground is a species of rattan (*Calamus*); the large tree in the background is *Anisoptera vidaliana. Anisoptera-Strombosia* formation.

VIII. View of a low, rocky terrace easily flooded. Along the edge of the stream at the right is *Homonoia riparia,* a willow-like euphorbiaceous shrub; next it, in the center of the photograph, are *Pandanus luzonensis* and the grass *Saccharum spontaneum.* The trees in the background, beginning from right to left, are *Dysoxylom schizichitode, Anisoptera vidaliana,* and *Sarcocephalus cordatus.* Photograph taken near close of the dry season. During the wet season this terrace is frequently flooded. *Anisoptera-Strombosia* formation.

IX. View on a river terrace 5 meters above low water. This terrace is never flooded. Trees present on left of the trail are *Dipterocarpus vernicifluus, Grewia stylocarpa, Cyathocalyx globosus;* on the right *Cyathocalyx, Ellipanthus luzoniensis, Shorea concorta, Dipterocarpus vernicifluus.* Note the abundance of bamboo, rattans, shrubby undergrowth and lianas. *Anisoptera-Strombosia* formation.

X. A terrace similar to that shown in Plate IX, with the undergrowth removed. Note the density of the forest, prevalence of low buttress roots, and the dead hanging lianas. *Anisoptera-Strombosia* formation.

XI. View on a terrace similar to that shown in Plate X. Note the prevalence of trees with fairly regular trunks. *Anisoptera-Strombosia* formation.

XII. Another view of a river terrace where the forest has an uneven profile. Note the large dominant *Dipterocarpus vernicifluus* with a tall buttress root. *Anisoptera-Strombosia* formation.

XIII. View across a banana field, showing in the background the irregular profile of a part of the *Anisoptera-Strombosia* formation.

XIV. View on a slope with a gradient of about 15 degrees. Passing from left to right are the following trees: *Anisoptera vidaliana* (with crooked trunk), the anonaceous *Xylopia dehiscens, Shorea polysperma* (large tree near the center), *Gnetum gnenom* (well in the background), and *Xanthophyllum* sp. Note the prevalence of lianas (principally *Agelæa wallichii*), rattans, and fairly good reproduction. *Anisoptera-Strombosia* formation.

XV. Vegetation on a slope of 35 degrees. Note the absence of many large trees and presence of rattans. Trees are *Strombosia philippinensis, Gnetum gnenom, Aporosa symplocosifolia, Cyclostemon bordenii,* etc. *Anisoptera-Strombosia* formation.

XVI. Vegetation in an open place where a species of *Calamus* (limoran) forms a dense growth. *Shorea-Plectronia* formation.

XVII. A species of *Ficus* or "balete" inclosing the trunk of *Crypteronia cumingii.* Note the large root on the right. *Shorea-Plectronia* formation.

XVIII. Shows the character of the bark of *Pithecolobium acle;* also a young plant of *Schefflera venulosa* starting in the soil accumulated in a knot hole. This latter species has the "balete" habit. *Anisoptera-Strombosia* formation.

XIX. A species of *Ficus* enveloping a tree of *Bischofia trifoliata. Shorea-Plectronia* formation.

XX. Shows the characteristic, net-like growth of a species of *Ficus* on *Santiria nitida. Anisoptera-Strombosia* formation.

XXI. View in the interior of a forest of the *Shorea-Plectronia* formation. Note the large buttresses of *Eugenia glaucicalyx.* Large tree on the right in the background is *Shorea polysperma.* Other trees shown are *Plectronia viridis* (several specimens on the right), *Cinnamomum mercadoi, Palaquium tenuipetiolatum, Thea montana, Memecylon edule,* and *Ternstrœmia toquian.* Note the scarcity of undergrowth and forest litter, and the presence of crustaceous lichens on the trunks of the trees. Photograph taken in Plot A, Table XIX.

XXII. View in the interior of a portion of the *Shorea-Plectronia* formation on a slope of 25 degrees. On the left is a large tree of *Eugenia glaucicalyx;* to the right of it is *Shorea polysperma;* between them in the background is *Calophyllum whitfordii.* (For other trees in this plot see Plot B, Table XIX.) The herbaceous vegetation is represented by *Carex continua.*

XXIII. Shows the bark characters and many low buttress roots of *Quercus bennettii*. Other trees present: *Shorea polysperma*, *Thea montana*, *Symplocos oblongifolia*, and others. *Shorea-Plectronia* formation.

XXIV. View into interior of a portion of the *Dipterocarpus-Shorea* formation. The three large trees are *Dipterocarpus grandiflorus*, *Gonocaryum tarlacense* is in front of the one on the left. Note the absence of buttress roots and the presence of a deep litter of leaves. Rattans are also present.

XXV. Base of the trunk of *Agathis philippinensis*, showing character of the bark and the exudation of the resin known as almaciga. (Gum copal.) *Shorea-Plectronia* formation.

XXVI. Eroding bank of the Lamao River. In the background is the tree fern, *Alsophila contaminans*. On the left is *Macaranga bicolor* and below it is *Ficus minahassæ*. On the right is a large tree of *F. minahassæ*, showing the hanging flower stalks originated on the trunk or the base of the larger branches. At the base of the tree fern are *Begonia rhombicarpa*, the fern *Polybotrya appendiculata*, and species of *Selaginella*. *Anisoptera-Strombosia* formation.

XXVII. Portion of terrace shown in Plate X, taken sixteen months later. All the undergrowth present started from seed. Note the presence of the large-leaved *Macaranga mappa*; also *Macaranga bicolor* and *Homalanthus populneus*, all these species being characteristic of recent clearings. *Anisoptera-Strombosia* formation.

PLATE I.

PLATE [illegible].

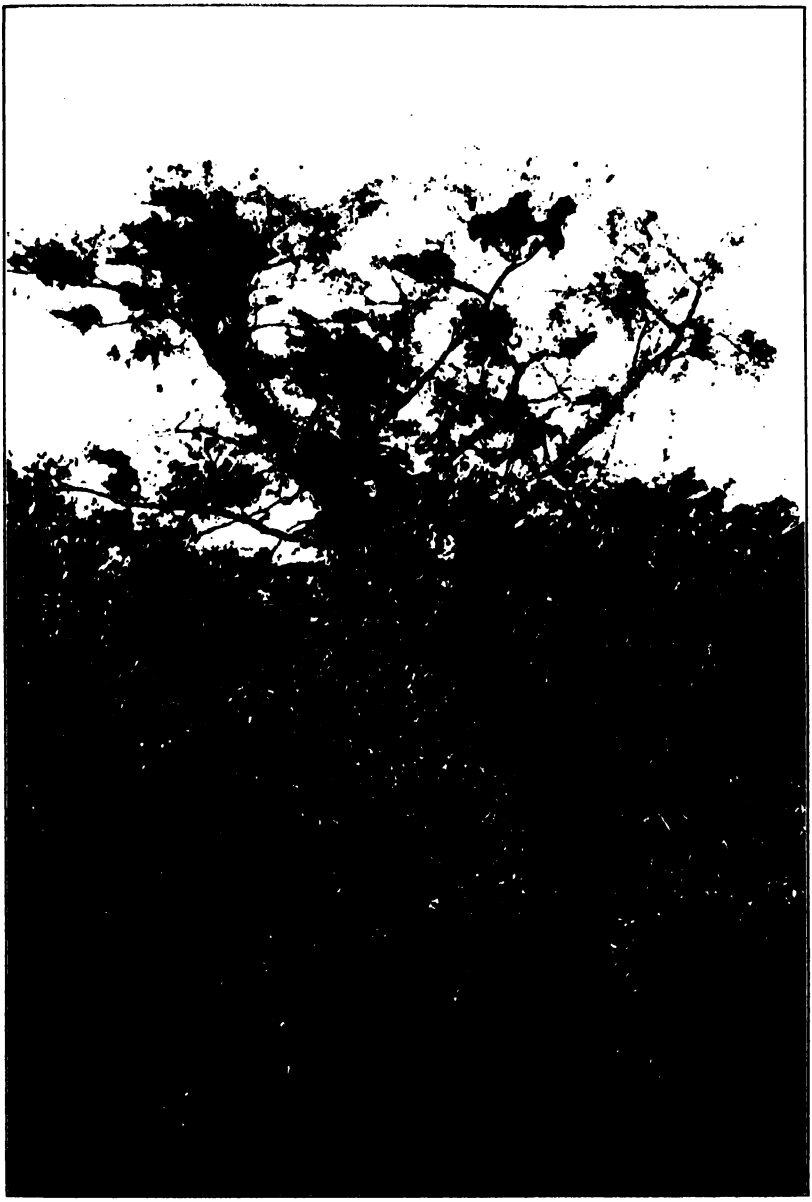

PLATE III.

PLATE IV.

PLATE V.

PLATE VI.

PLATE VII.

PLATE VII.

PLATE IX.

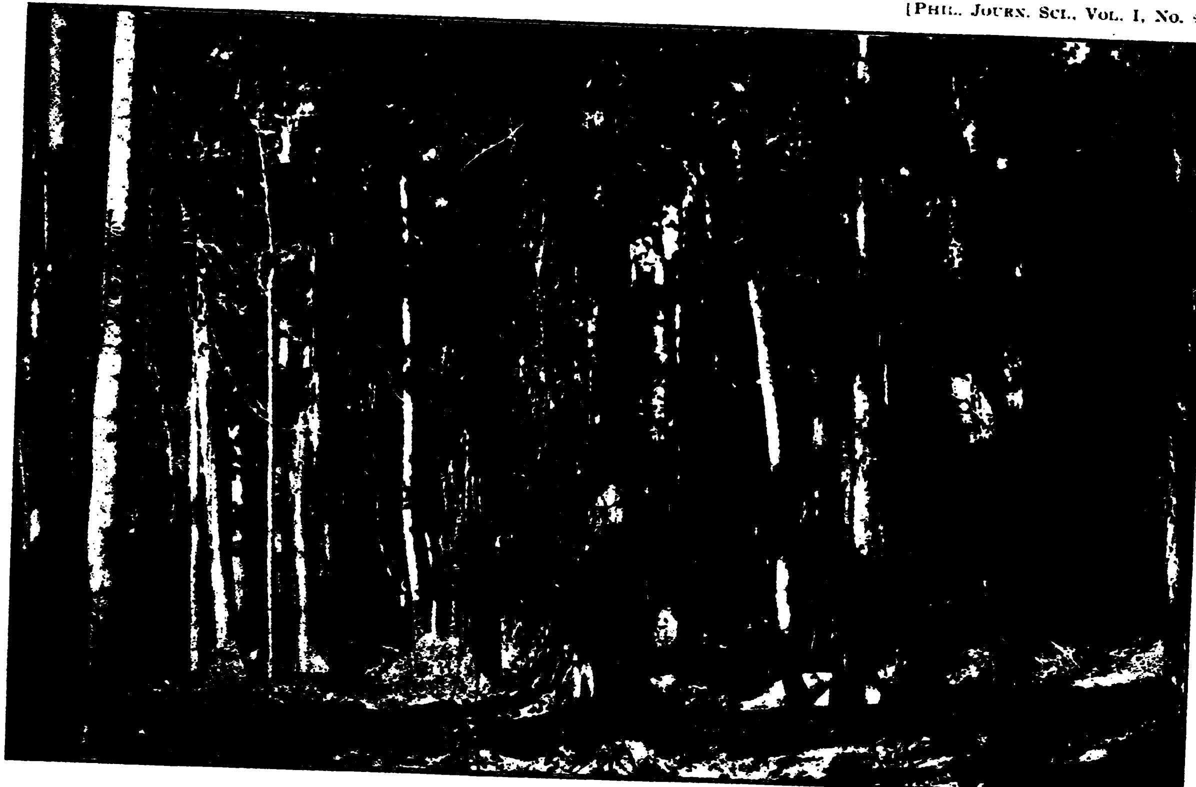

PLATE X.

PLATE XI.

PLATE XII.

PLATE XIII.

PLATE XIV.

PLATE XV.

PLATE XVI.

PLATE XVII.

PLATE XVIII.

PLATE XIX.

PLATE XX.

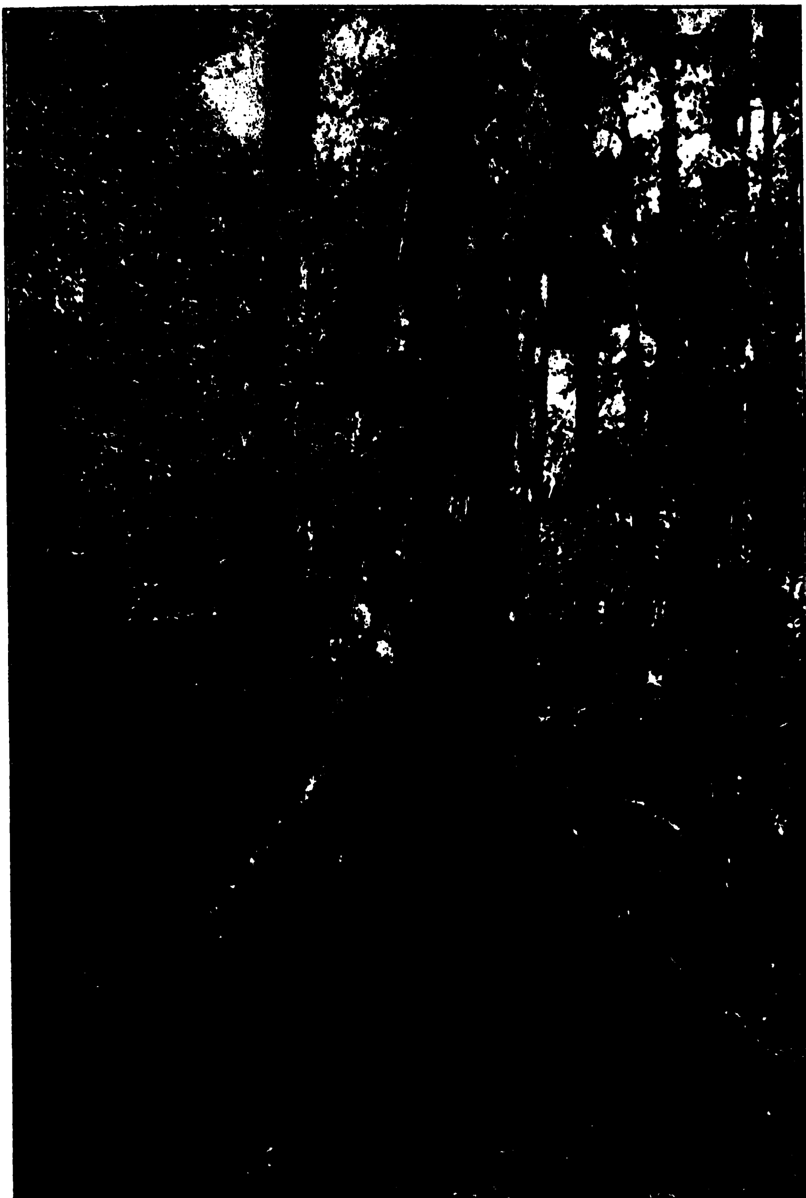

PLATE XXI.

PLATE XXII.

PLATE XXIII.

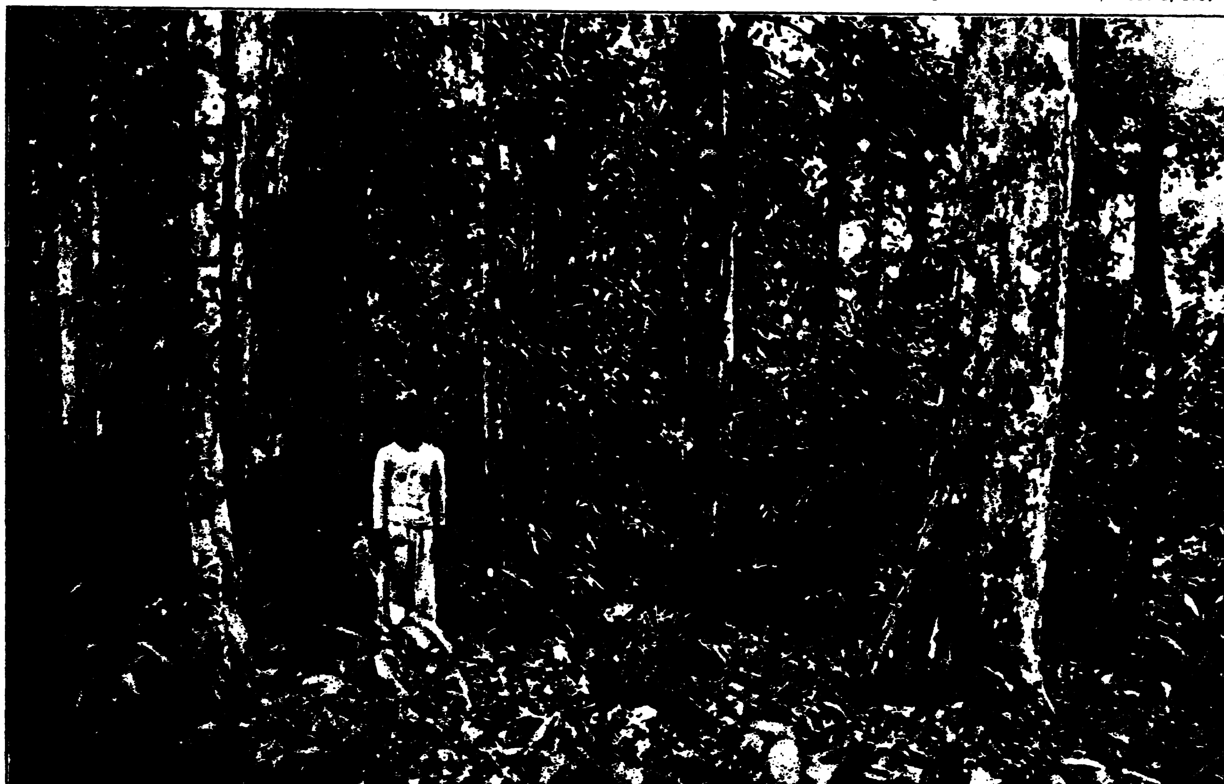

PLATE XXIV.

PLATE XXV.

PLATE XXVI.

PLATE XXVII.

THE WATERS OF THE CRATER LAKES OF TAAL VOLCANO WITH A NOTE ON SOME PHENOMENA OF RADIO-ACTIVITY.

By R. F. BACON.

(*From the Chemical Division, Bureau of Science.*)

The crater of Taal Volcano, in the Province of Batangas, central Luzon, has in it two very remarkable pools. They are known as the Green Lake and the Yellow Lake, respectively, owing to the color of their waters. About 2 liters of water was obtained from each of these by the members of an expedition from this laboratory, who visited the volcano during the present dry season (January, 1906).

The volcano consists of a series of elevations containing, among others, a large crater about 3 miles long and 2 miles wide, rising from the waters of Lake Taal. Within this is a small crater, at present active in the solfotaric stage, and the ash cones of several dormant ones. There are also three lakes within the boundaries of the large extinct crater; two of these have been mentioned above, the other is in the crater of the present active cone and it was not accessible at the time of this expedition. The water of this lake is at a boiling temperature and large volumes of steam continually rise from its surface; the temperature of the other two, at the time of the visit, was only a little above that of the air (about 38° C.). These lakes are described as being boiling hot by former investigators and there is little doubt but that they may change very markedly from season to season.

These waters were considered to be deserving of investigation because, as they possibly had such free access to the depths of the earth, they might be of an extraordinary composition. The analyses proved that, while the concentration and acidity of the waters were remarkably high, rare or unusual elements could not be detected in the amount of liquid available, neither were we able to demonstrate that the samples were radio-active, but this lack of radio-activity may be due to the fact that they had been standing for about four weeks before it was possible to make the necessary tests. A rock sample obtained from the yellow pool was not radio-active. The determinations were made by dissolving a portion of this sample in acid and then drawing air through the solution and into a gold-leaf electroscope, the construction of which is described below. This specimen was a very porous basalt which had soaked up a large quantity of water, and on standing in the laboratory it gradually

became covered with a growth of alum crystals which were colored yellow by a small amount of ferric salt. In appearance the crystals deceptively resembled those of sulphur, and there is no doubt but that many statements concerning the large amount of sulphur in volcanic craters would be more accurate if iron salts were taken into consideration. The negative results obtained from this rock make it appear probable that there are no radio-active salts in solution in the water.

Water No. 1 was of a decidedly green color and when brought into the laboratory was comparatively free from sediment; No. 2 was of a greenish-yellow and was partially filled with a sediment which largely consisted of gypsum. No odor of hydrogen sulphide or of sulphur dioxide was perceptible in either water and microscopic examination revealed no organized matter.

Qualitative analysis showed the presence of ferrous and ferric iron, ammonium, aluminum, phosphoric acid, magnesium, calcium, potassium, sulphuric and chlor-ions, barium and strontium (spectroscopic determination only). No cæsium or rubidium could be detected.

The quantitative analysis is as follows:

Quantitative analyses of waters from the crater of Taal Volcano.

[Figures express grams per 100 cc. of the water.]

	No. 1.	No. 2.
Color	Green.	Yellow.
Sp. gr. at 15° C.	L 1062	1.1763
Acidity	1.28 N.	2.08 N.
Acidity calc. as H_2SO_4	6.14%	10.28%
Calc. as HCl	4.65%	7.57%
Total solids (filtered water), by heating to 110° for 6 hrs.	15.8678	25.7235
Total solids (unfiltered water), by same method	15.9312	30.3175
(Sediment difference)	.0634	4.5940
SO_4	4.1303	5.6768
Cl	6.3143	11.2760
Fe (total)	1.031	1.711
$\ddot{Fe}$	.7150	1.3250
$\dddot{Fe}$	.3160	.386
Al	1.1892	3.485
Na	1.7217	.9343
PO_4	.0273	.0391
Ca	.0934	.1042
Mg	.9432	1.8210
K	.0418	.0514

The following is the analysis made by José Centeno[1] in 1885, calculated

[1] José Centeno, "*Estudio Geológico del Volcán de Taal*" 21, 22. Also in Becker "*Reports on Geology of the Philippine Islands*", p. 49. Extracts from *21st Annual Report U. S. Geol. Survey* (1899–1900), Part III.

as ions, the results expressed in grams in 100 cc. water. No. 1 is described as the yellow or more northerly lake and No. 2 as the green one.

	No. 1.	No. 2.
Total solids	2.6988	6.0022
$\overset{\cdots}{Fe}$	.1315	.3036
$\overset{\cdot\cdot}{Fe}$	.0209	.0612
Na	.6471	1.2405
K	.0372	.1821
Cl	1.2873	3.4596
SO_4	.4122	.4186
PO_4	.0396	.0515
Al	.0143	------
Mg	.0264	.0335
Ca	.0150	.0138
Free acid calc. as H_2SO_4	.15%	
Free acid calc. as HCl		.894
SiO_2	.0640	.074

A comparison of these two analyses with the recent ones from this laboratory shows a marked difference; the water we obtained is much more concentrated and the content of free acid is higher.

In connection with the study of the radio-activity of these waters, some very curious phenomena were noted, of which it is desired to make a preliminary announcement at this time. The general arrangement of the apparatus we used was that of H. Mache.[2] In place of the large bell-jar containing the electroscope, a Novy anaërobic jar was used. In this was constructed an electroscope similar to that described by Boltwood.[3] Behind the gold leaf was placed a photographed scale which was magnified by a suitable arrangement. In ordinary, diffuse light in the middle hours of the day, the rate of discharge of the electroscope was very constant, the gold leaf moving one division of the scale in five minutes. However, it was noted that late in the afternoon the rate became much slower, being one division in ten to fifteen minutes. In the sunlight, it was found that the discharge rate was very markedly increased, averaging 18 divisions in five minutes. Experiments in total darkness gave a total discharge rate of 1 division in two hours. These experiments in each case extended over a period of from thirty minutes to four hours and the rate was then calculated to a unit time of five minutes; however, readings were generally made every five minutes, and for given conditions the rate was usually quite uniform throughout a series. We have also noted a singular behavior in uranium salts placed in the sunlight. The air passed through an aqueous solution of a uranium salt after the latter had been exposed to sunlight for a short time is about twice as active in discharging the electroscope as it is from the same solution kept in diffused light, this acquired activity being rapidly lost

[2] *Monatshefte für Chemie* (1905), 26, 595.
[3] *Am. Jour. of Sci.* (Sill.) (1904), 18, 97.

on removing the salt from sunlight. This result is not surprising in view of the work of Seekamp [4] and others in which they demonstrated the powerful catalytic action which uranium salts displayed only while in the sunlight.

The general action of sunlight is often such as to make chemical substances better conductors of electricity.[5] Thus, in the sun's rays, sulphur, phosphorus, selenium,[6] and mercuric sulphide [7] are changed into modifications which more easily conduct the electric current. Silver [8] and gold [9] oxides lose and lead oxide [10] and manganous oxide [11] gain oxygen, each to give a substance which offers less resistance to the passage of the current. In aqueous solution the same facts are noted. Chlorine and bromine water give the respective hallhydric acids; an aqueous solution of sulphur dioxide,[12] even in the absence of free oxygen, is changed in the sunlight into sulphuric acid and sulphur; a solution of potassium iodide gives the better conducting potassium hydroxide;[13] ferric chloride with oxalic acid and with alcohol [14] gives a mixture with increased conductivity, and the same is true of mercuric chloride in conjunction with ammonium oxalate.[15]

Hertz [16] showed that a spark is formed much more easily in air when the spark gap is in the light than when it is in the dark, and Arrhenius [17] from his own work and that of Hertz on the conductivity of gases at low pressure under the influence of light makes the deduction that light has a tendency to ionize the air and thus to make it a better conductor. Mrs. Ayrton [18] among many curious experiments demonstrated that a candle flame could discharge an electroscope very rapidly at a distance of 40 centimeters. Many more instances can easily be added to this list. We believe there is here a more general relation which has not as yet been explained and therefore we expect to study these interesting phenomena in a much more exhaustive manner. For this purpose we have ordered apparatus of sufficient delicacy, but at the time of writing the proper instruments are not available in the Philippines, and for this

[4] *Ann. d. Chemie* (Liebig) (1862), **122**, 113; (1865), **133**, 253.

[5] See J. Gibson, *Ztsch. Phys. Chem.* (1897) **23**, 349. Arrhenius, *Wien Akad.* (1887) **96**, 831.

[6] *Proc. Roy. Soc.* (1889) **46**, 136.

[7] Heumann, *Ber. d. chem. Gesell.* (1874) **7**, 750. Böttger, *Chem. Centralbl.* (1875) 291.

[8] Arrhenius, *Wien. Akad.* (1887) **96**, 831.

[9] Chevreul, *C. R.* (1858) **47**, 1007.

[10] Schonbein, *Edmann's Journal* (1850) **51**, 273.

[11] Chastaing, *Ann. Chim. Phys.* (1876) (5) **11**, 145.

[12] Idem, *l. c.*

[13] Pogg. *Ann.* (1863) **119**, 497.

[14] Chastaing, *l. c.*

[15] *Wien Akad.* (1880) **80** (2) 636.

[16] Hertz, *Wied. Ann.* (1887) **31**, 983.

[17] Arrhenius, *Wied. Ann.* (1888) **33**, 643.

[18] *Nature* (1902) **65**, 390.

reason we beg to reserve the field for a short time. We expect in our next communication to be able to report more authoritatively on the radio-activity of the Taal waters, including those of the lake inside the present active cone, and an expedition will probably be organized to obtain samples before the eruptions attendant upon the rainy season may possibly have destroyed the lake. We believe such observations will be of value in judging certain theories of the present day concerning the radio-activity of the surface of the earth. For the sake of completeness an analysis of the water of the larger lake surrounding the volcano, which was made some two years ago by Mr. F. A. Thanisch, formerly of this Bureau, is appended.

[Results expressed as parts in 100 cc.]

Total residue	0.1716
Fixed residue	0.1484
Loss on ignition	0.0232
Free NH_3	Trace.
Nitrites	Traces.
Fe_2O_3 } Al_2O_3 }	0.00042
Ca	0.005339
Mg	0.004942
Na	0.045634
SiO_2	0.00315
Cl	0.0720
SO_4	0.01913
CO_2, as bicarbonate.	Considerable.

The water has a slightly alkaline reaction and contains a slight white deposit.

The above demonstrates the radical difference between the waters of the lakes within the crater and of that of the lake surrounding Taal Mountain.

CONCERNING SILVER OXIDE AND SILVER SUBOXIDE.

By Gilbert N. Lewis.
(*From the Chemical Division, Bureau of Science.*)

On account of the uncertainty as to the correctness of the value at present accepted for the electrolytic potential of oxygen, I have attempted to calculate this extremely important quantity by an indirect method. One very necessary datum needed in this calculation is the decomposition pressure of silver oxide at 25°. The determination of this pressure is the subject of the present paper. Incidentally it will be necessary to consider the suboxide of silver which, by certain chemists, has been supposed to exist.

LeChatelier[1] was the first to show the reversibility of the reaction,

$$2\ Ag_2O = 4\ Ag + O_2.$$

By the decomposition of silver oxide in a closed tube at 300° he obtained a pressure of 10 atmospheres. On the other hand, by heating silver at the same temperature in oxygen at 15 atmospheres he observed the oxidation of the silver. He therefore placed the decomposition pressure of silver oxide between 10 and 15 atmospheres.

Knowing this pressure for one temperature, and the heat of decomposition, it should be possible to calculate with the aid of the van't Hoff equation, the pressure at another temperature. However, such a calculation must be made with great caution. In the first place, we must be sure that at both temperatures we are dealing with precisely the same reaction.

Guntz[2] has undertaken to show that the pressure he obtained by heating silver oxide at 358° was not the decomposition pressure of silver oxide but of silver suboxide. In other words, he believed that silver oxide at first decomposed entirely into suboxide and oxygen, according to the equation,

$$4\ Ag_2O = 2\ Ag_4O + O_2,$$

and that the suboxide then partially decomposed until equilibrium was reached, according to the equation,

$$2\ Ag_4O = 8\ Ag + O_2.$$

[1] *Zeit. Phys. Chem.* (1887), 1, 516.
[2] *C. R.* 128 (1899), 996.

The existence of such a suboxide would not prevent the determination of the equilibrium pressure in the system, Ag, Ag_2O, O_2 for this pressure could readily be calculated from the equilibrium pressure in the system Ag_2O, Ag_4O, O_2, and that in the system Ag_4O, Ag, O_2. However, I shall show that in experiments covering a wide range of temperature, the suboxide never appeared and that the experiments which led M. Guntz to believe in its existence are probably to be interpreted otherwise.

I shall first describe measurements of the decomposition pressures at several temperatures and then show that the quantities thus obtained represent true equilibrium in the system Ag_2O, Ag, O_2.

Two samples of silver oxide were used. These will be referred to as A and B. A was prepared from hot, dilute barium hydroxide and silver nitrate as described in my paper on the kinetics of the silver oxide decomposition, carbon dioxide being excluded.[3] B was the preparation of Merck, labeled "highest purity." Both samples before being used were heated for several hours at 300°.

DECOMPOSITION PRESSURE AT 325°.

The first experiment was made at 325° [4] in the thermostat of molten sodium and potassium nitrates which I have described in the paper just mentioned. The apparatus for measuring the pressure consisted of a stout, brass tube, about 2 meters long and 1 centimeter in internal diameter, closed at the lower end and at the upper connected with a metallic manometer. In the lower end of the brass tube was fitted a thin, glass tube 7 cm. high containing the silver oxide (Sample B). In order as far as possible to diminish the total air space in the apparatus, the rest of the brass tube was filled as nearly as possible with a close fitting glass rod. After the apparatus had been exhausted through a stopcock provided for this purpose the lower end of the tube was introduced into the thermostat to a depth of about 30 cm.

The decomposition began in a few hours and in five days the pressure reached a constant maximum as shown in Table I.

TABLE I.

Time in hours.	Pressure in atmospheres.
12	18.5
24	26.5
48	29.8
72	30.1
96	31.5
120	31.8
144	31.8

[3] *Zeit. Phys. Chem.* (1905), 52, 310; *Bulletin of the Bureau of Government Laboratories, Manila*, No. 30.

[4] All thermometers used were compared with Reichsanstalt standards. The corrected temperatures are given.

In order to approach the equilibrium from the other side the tube was then heated for a few hours at a higher temperature and returned to the thermostat. The pressure at first registered 34.0 atmospheres and then fell gradually as shown in Table II.

TABLE II.

Time in hours.	Pressure in atmospheres.
0	34.0
24	33.4
48	32.5
72	32.3

According to these data the true pressure of equilibrium lies between 31.8 and 32.3. The manometer had been verified by comparison with a standard manometer but may have been in error by a considerable fraction of an atmosphere. Therefore, we will take in round numbers 32 atmospheres as the decomposition pressure at 325.°

During this whole experiment there was not the slightest leakage from the tube and the whole apparatus was eminently satisfactory. Unfortunately, the work was interrupted at this point by my removal from the chemical laboratory of Harvard College to the Government Laboratory of the Philippine Islands and the experiments had to be continued with other apparatus.

THE DECOMPOSITION PRESSURE AT 302°.

The next experiments were made in a bath of diphenylamine vapor. The first method used was an indirect one. It consisted in heating the silver oxide in a closed glass tube which was drawn out at one end to a capillary; cooling it suddenly by removal from the bath; opening the capillary under a gas burette; and calculating from the volume of gas thus collected and from the volume of the tube (exclusive of that occupied by the remaining mixture of silver and oxide) the pressure which had been exerted in the tube at the temperature of the bath. Eight tubes containing different quantities of silver oxide were suspended in the vapor of boiling diphenylamine contained in a large iron receptacle, and were kept in this bath for six days. During this time it was found necessary to renew the diphenylamine several times on account of its gradual decomposition. The pure substance (Kahlbaum's) boiled at 302°. In the course of the experiment several of the tubes were broken; in others the oxide had entirely decomposed, so that finally only one tube of the eight could be used for determining the pressure. This was broken under water and 8.58 cc. of oxygen was collected in the burette at 760 mm. and 30°. The tube was weighed, then filled with ether, boiled to drive out all the air, filled again and weighed. From these two weighings the volume of the tube was found to be 0.92 cc. The pressure at 302° must therefore have been $\frac{8.58+0.92}{0.92} \cdot \frac{273+302}{273+30}$, or 18.0

atmospheres. It will be shown presently that this value is too small. The oxide used in this tube was Sample A, which decomposes, as I have shown, far more slowly than B. It is probable that equilibrium had not been established when the experiment was ended. On account of this difficulty of determining when the reaction had come to equilibrium, the indirect method was abandoned and the following adopted:

Two tubes, one having a bore of about 0.7 mm., the other of 3mm., were joined together and then drawn apart at the junction to a fine capillary about 1½ m. long (BCD in Fig. 1). In the smaller tube D, a short column of mercury was introduced, and the open end sealed, thus making an ordinary closed manometer, which was calibrated in the usual way after the conclusion of the experiment. The tube B was filled with silver oxide and the open end sealed.

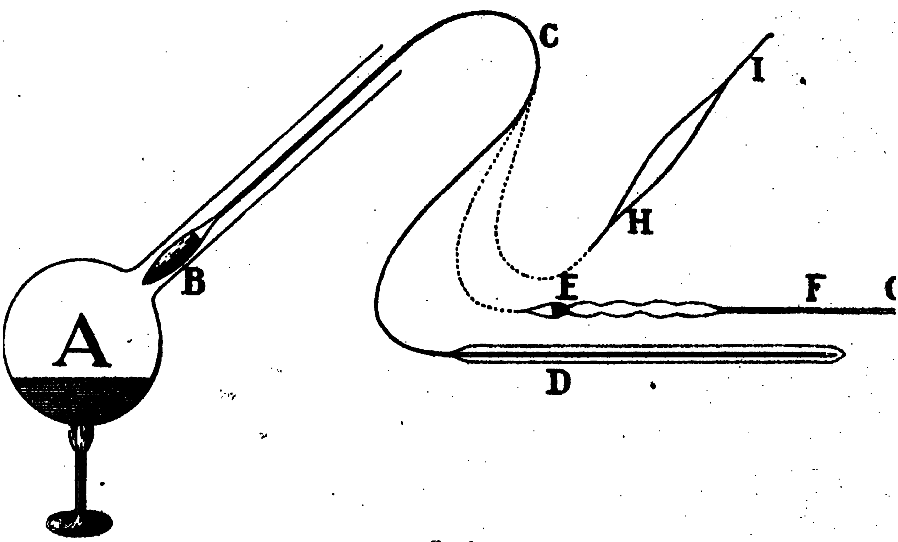

FIG. 1.

The large bath used in the preceding experiment had also to be abandoned on account of the great consumption of dyphenylamine, of which only a limited supply was available. A simple bath consisting in a long-necked glass flask was found to answer the purpose. The neck of the flask was about 2 cm. in diameter and was insulated externally by a coat of asbestos. The temperature in different parts of the neck was frequently tested and the variation over a considerable range rarely amounted to half a degree. The tube B was held in a simple framework of glass which prevented it from touching the sides of the flask.

In the preceding paper I have shown that the decomposition of silver oxide is extremely slow until a large amount of silver has formed, which acts as a catalyzing agent. Therefore, in order to hasten the attainment of equilibrium, the tube at the beginning of the experiment was heated to a higher temperature than that of the bath until a small part of the

oxide was decomposed. The tube was then introduced into the bath and heated continuously, except that at the end of each day it was removed and the diphenylamine renewed. After being replaced in the bath the pressure always returned at once to its previous value, thus showing that during the rapid cooling and heating the reaction had not proceeded to any appreciable extent. During the experiment the temperature varied, chiefly on account of the gradual decomposition of the diphenylamine, between the 301.5° and 303°. We may take 302° as the average temperature. It may be noted here that 1° difference in temperature makes a difference in pressure of a little less than half an atmosphere.

The first experiment was made with sample B of the oxide. After being inclosed in the tube it was heated gently with a Bunsen flame until white spots began to appear in it. It was then introduced into the bath, the manometer indicating 6 atmospheres. At the end of two days the pressure had reached 16.7 atmospheres and was increasing very slowly. The tube was then removed from the bath and heated until a little more of the oxide was decomposed. When replaced in the bath the pressure was 20.2 atmospheres. During the next twenty-four hours the pressure remained constant at this point within one-tenth of an atmosphere. The tube was again removed and heated until, on being replaced, 23.2 atmospheres was registered. In the next twenty-four hours this pressure fell to 21.6 and in twenty-four hours more to 21.5 atmospheres.

This experiment shows that the decomposition pressure at 302° must be considerably above 16.7 atmospheres (contrary to the conclusions of LeChatelier, who placed it between 10 and 15 atmospheres), it is probably not far from 20.2 and it certainly is not greater than 21.5.

The experiment was then repeated with another tube containing the same kind of oxide. In this case a longer time was allowed for equilibrium to become established. Table III shows the pressure at the end of each twenty-four hours.

TABLE III.

Time in days.	Pressure in atmospheres.
1	11.1
2	15.1
3	17.3
4	18.8
5	19.5
6	19.7
7	19.7
8	19.7

It is evident from these two experiments that the correct pressure must lie between 19.7 and 21.5, and probably nearer to the former value than to the latter. Let us take 20.5 as a value sufficiently exact for our purposes.

THE DECOMPOSITION PRESSURE AT 445°.

The next experiments were made in sulphur vapor, the bath being arranged as in the last experiment. At this temperature also, the first attempts were made with an indirect method. The simple method previously used seemed hardly likely to succeed here, as a change of equilibrium in the tube would probably occur during the process of cooling from the temperature of boiling sulphur. A new method was therefore adopted. The construction of the apparatus is indicated in fig. 1 by B C H I. A thick-walled, glass tube of not more than 2 mm. internal diameter was drawn out to form a long capillary C. The tube B on one side of the capillary was filled with silver oxide and closed at the free end. The tube was again drawn to a capillary at I and sealed, leaving about 3 or 4 cm. between H and I. During this construction a small piece of tar was placed loosely in the tube where it narrowed to a capillary at H. When the apparatus had been in the bath long enough for equilibrium to be established a hot glass rod was brought for a moment in contact with the tube at H, thus melting the tar and causing it to run into the capillary. This heating was purely local and had no appreciable effect on the temperature of the gas in the tube H I. After the tar had solidified the capillary was broken at C leaving in the tube H I the original pressure. The capillary I could then be opened under water and the pressure calculated from the volume of gas evolved and the volume of the tube H I.

While this method was being used, experiments were also made with a direct method, a manometer being finally constructed which gave very satisfactory results. It is shown as E F G in fig. 1. B C E F G was drawn from a single tube of the kind used in the preceding method. At E it was drawn into a series of small bulbs, as these proved to be stronger than a single straight tube, and finally it terminated in a long capillary which had a nearly uniform bore (about 0. 1 mm.) in the region F G. The pressure was registered by a small mercury column standing at E at atmospheric pressure, and at a point F, about 10 cm. from the end of the capillary, when the pressure reached its maximum. On breaking the tube at C after the experiment, the mercury column invariably returned to its initial position at E. The capillary point at G was then broken off within one or two tenths of a millimeter from the end, and the volumes F G and E G were determined by drawing in mercury successively to F and to E and weighing the quantities drawn in. The smaller amount being only a few milligrams was weighed on a fine assay balance. I believe that this manometer gives pretty accurate results. The error caused by the capillarity of the mercury is not over one-tenth of an atmosphere, nor is it likely that the change in volume of the capillary tube when the pressure is exerted from within is large enough to cause any great error.

Two difficulties were met in the use of both the methods which I have described. At the high pressure of about 200 atmospheres which is here reached, the glass tubes are likely to explode upon the slightest provocation. Fortunately, although explosions in the external tube were frequent, the tube B within the bath rarely gave way. This difference between tubes of the same form and drawn from the same piece of glass is doubtless to be attributed to the annealing and toughening of the glass at the temperature of the bath. The danger of explosion in the outer tube was reduced as far as possible by choosing flawless tubes and drawing them with care. The capillaries never burst and probably could have withstood a much greater pressure than that to which they were subjected. On account of their flexibility and strength they form an excellent means of communicating high pressures.

The other difficulty was that ordinarily enough silver oxide could not be packed into the tube B to give the pressure of equilibrium before being entirely decomposed. This difficulty was overcome by filling only one-half of the tube with silver oxide, the rest being filled with powdered potassium permanganate. The latter decomposes at once at the temperature of the bath and produces a much larger quantity of oxygen than the same volume of silver oxide. The two substances were separated by a small piece of asbestos.

Experiments in which the manometer was used showed that equilibrium was reached in from fifteen to forty minutes, according to the kind of oxide used. In one case an addition was made to the apparatus in an extra bulb at C' filled with potassium permanganate. After the manometer had come to rest as usual, the bulb at C' was gently heated causing a further increase of pressure, but this excess of pressure rapidly disappeared through the reoxidation of the silver in B and within ten minutes the pressure was the same as before, within a small fraction of an atmosphere. This shows that we are dealing here with a case of true equilibrium.

The first experiments with both methods gave disappointing results. The pressures calculated with the aid of Boyle's law, ranged between 175 and 200 atmospheres. Those obtained by the indirect method were on the average several per cent higher than those obtained with the manometer. However, this difference disappears when allowance is made for the difference in compressibility[5] of air and oxygen contained respectively in the tubes EG and HI.

A more careful investigation of the temperature in the neck of the flask pointed to the cause of the observed variations in pressure. Although a preliminary test had shown the temperature to be the same in the neck and the body of the flask this proved not to be the case always. The

[5] Amagat, *Ann. de Chim.* (5) (1880), **19**, 345; (5) (1881) **22**, 353.

temperature in the neck was found to vary considerably when the conditions, such as the angle at which the flask is held, and the height of the heating flame, were changed. This difference between the sulphur and diphenylamine baths is probably not so much due to the higher temperature of the former as to the great viscosity of the sulphur condensing in the neck of the flask.

The tube containing the silver oxide had been placed in the neck rather than in the body of the flask in order to avoid possible variations of temperature due to spattering of the superheated liquid. Upon experiment, however, this superheating proved to be small. The Reichsanstalt standard thermometer showed the temperature of the liquid to vary under varying conditions between 445° and 445.5°, the temperature in the body of the flask between 443.5° and 444.5°. Since the thermometer may have been in error by 1 or 2 degrees (it was calibrated at 397° and 505°) we will use, as the temperature in the body of the flask, 445°, the accepted value for the boiling point of sulphur.

Four new experiments were now made similar to those already described except that the tube B containing the oxide was now placed entirely in the body of the flask. The direct method with the manometer was used.

For illustration I will give the details of the first of these experiments. The mercury column, moving rapidly at first, gradually came to rest. Its position was read on a scale attached to the capillary. Table IV gives the distances of this column from the end of the manometer at intervals of about five minutes. The experiment was begun at 10:25:

TABLE IV.

Time.	Distance in mm.
10.32	65.0
10.35	53.9
10.40	49.0
10.45	45.9
10.50	45.5
10.55	45.2
11.00	45.2
11.05	45.2
11.10	45.3
11.15	45.2
11.25	45.2

The apparatus was now broken at C and the manometer calibrated; 0.842 grams of mercury was required to fill the whole manometer, 0.00413 grams to fill the end of the capillary to the point (45.2 mm.) where the mercury came to rest. If we assume Boyle's law to be correct the pressure is $\frac{0.842}{0.00413}$, or 204 atmospheres.

The results of this experiment and those of others made under the same conditions are given in Table V.

TABLE V.

No. of experiment.	Kind of Oxide.	Pressure.
1	B	204
2	B	191
3	B	200
4	A	205

Experiment 2 differed from the others in that the silver oxide was almost entirely used up. The result is undoubtedly too low. Discarding this value therefore and taking the average of the other three the result is 203 atmospheres.

The preceding calculations have been based on the assumption that the air in the manometer obeys Boyle's law. According to the work of Amagat[6] the true pressure of air in the neighborhood of 200 atmospheres is, at 0°, about 2½ per cent higher than that calculated from the volume by Boyle's law. At 30°, the temperature of the manometer, this correction would be somewhat less, say two per cent. Making this correction, therefore, we get 207 atmospheres as the equilibrium pressure of silver oxide at 445°.

DOES SILVER SUBOXIDE EXIST?

M. Guntz[6] heated silver oxide to a temperature of 358° for fifty hours and obtained a pressure of 49 atmospheres. This he considered the pressure of equilibrium in the reaction

$$2\ Ag_4O = 8\ Ag + O_2$$

and cited as evidence for this view the following experiment.

In a closed tube were placed known weights of silver and silver oxide and a quantity of potassium permanganate sufficient to yield enough oxygen to produce a pressure higher than 49 atmospheres at 358°. The whole was heated at this temperature for three days. The tube was then cooled rapidly and it was found that the silver had gained in weight and the silver oxide had lost in weight by amounts which indicated the formation of Ag_4O in both cases.

I shall presently show that in all my experiments carried on at temperatures from 300° to 445°, and with two different samples of silver oxide, the suboxide of silver was never formed. It seems difficult to reconcile my results with those of M. Guntz. Of course, it is conceivable that the suboxide is capable of existence and that it appeared for some reason in one case and not in the other, just as occasionally some hydrate may be suddenly precipitated from an aqueous solution although it may fail to appear many times under apparently similar circumstances. However, I am inclined to believe that the experiments of M. Guntz can be explained without the necessity of assuming the existence of the suboxide. If the pressure in the tube that he used was greater than the

[6] L. c.

decomposition pressure of silver oxide, the gain in weight of the silver can be readily explained by its oxidation to silver oxide. The loss in weight of the silver oxide can only be explained by assuming that it contained water or carbon dioxide. I should consider this improbable if it were not for the fact that my own experiments have shown how difficult it is to prepare and to keep silver oxide free from these impurities. It is perhaps not generally known that carbon dioxide, as well as water, is rapidly taken up by silver oxide from the atmosphere. One sample which I prepared in such a way that it was absolutely free from this impurity absorbed a considerable quantity of carbon dioxide while standing for a month in a loosely corked bottle. When carbon dioxide and water are once present, they can be expelled only with great difficulty. Some of the former frequently remains after the silver oxide has been heated an hour or two at 300°.

That silver suboxide was not present in any of the experiments I have described will be evident from the following considerations. According to the phase-rule, silver, silver oxide, silver suboxide, and oxygen can not all exist together in equilibrium. In the absence of suboxide only one state of equilibrium is possible, that between Ag, Ag_2O, O_2. But in case the suboxide could form, then two states of equilibrium would be possible, namely between Ag_2O, Ag_4O, O_2, and between Ag_4O, Ag, O_2. With a given quantity of silver oxide enclosed in a small volume the first equilibrium would exist; in a larger volume, the second.

In all the experiments previously described the final mixture in the tube has contained silver, as shown by the color and by the metallic flakes produced upon grinding the whole in a mortar. In any given case, therefore, the remaining black powder must be a single phase, either all silver oxide or all silver suboxide. Let us assume that it is the latter and see to what consequences we are led. For example, let us assume that the pressure of 32 atmospheres which we obtained at 325° is the decomposition pressure of silver suboxide. Now suppose that we inclose so much silver oxide in a tube that if it should all decompose according to the reaction,

$$4\ Ag_2O = 2\ Ag_4O + O_2$$

a pressure of over 32 atmospheres would result. Then metallic silver could not form and the only possible equilibrium would be that between the oxide and suboxide.

This very experiment was undertaken. A glass tube was filled with silver oxide, sealed, and heated for a week at 325°. At the end of this time a considerable quantity of silver had formed, although, calculated from the volume of the tube and the amount of oxide, over 100 atmospheres would have been generated by the change from the oxide to the suboxide alone. A similar experiment was made at 345°. A small quantity of silver was placed in a tube and covered with a little glass wool.

The rest of the tube was filled as full as possible with silver oxide. The tube was then sealed and left in the thermostat for five days, at the end of which time not only was the silver unoxidized but white spots of silver had appeared throughout the oxide.

These experiments show pretty conclusively the absence of silver suboxide. A still stronger proof is given by the experiments at 302°. In one of the experiments at this temperature which showed the pressure of equilibrium to be about 20 atmospheres, the volume of the tube and the manometer and the weight of silver oxide were determined. It was found that enough oxide had been used to produce a pressure of 35 to 40 atmospheres if it had all changed to suboxide, yet a considerable quantity of silver was found in the final mixture.

Finally, it was found possible to show by direct analysis that the black powder left in the tubes was not silver suboxide. It sometimes happened that at the end of the experiment the silver oxide was almost entirely decomposed in one part of the tube and apparently unchanged in another. This was due to the fact that the decomposition being autocalytic in character, progresses very rapidly at any point where it has once begun. In the first experiment at 302° a considerable portion of the black substance left in the tube appeared to contain no silver. A sample of this weighing 0.0892 grams was heated in a tube connected with a gas burette. It yielded 4.71 cc. of oxygen (at 27°, 760 mm.). The above quantity of pure silver oxide should give theoretically 4.73 cc. This is an unexpectedly good agreement.

In the experiments at 445° there was no case in which the remaining oxide did not contain some silver, but two samples were chosen which appeared to be least decomposed; 0.205 grams of the first gave 7.24 cc. of oxygen (calculated for Ag_2O, 10.85 cc.; for Ag_4O, 5.42 cc.); 0.0851 grams of the second gave 4.35 cc. (calculated for Ag_2O, 4.52 cc.; for Ag_4O, 2.26 cc.). It is obvious that here also the black substance can not be silver suboxide and is presumably silver oxide mixed with a certain amount of silver.

THE HEAT OF FORMATION OF SILVER OXIDE.

We have now obtained the pressure of equilibrium at different temperatures between silver, oxygen, and a certain black powder. We have proved that this black powder is not silver suboxide, but that at 302°, and presumably at the other temperatures as well, it has exactly the composition, Ag_2O. There still remains the possibility that this substance, although of the same composition as the silver oxide which exists at ordinary temperatures, may be an allotropic phase. In other words, there may be a transition temperature between room temperature and the temperatures of our experiments at which a break in continuity of the properties of silver oxide occurs. This possibility must be eliminated before we can safely calculate the decomposition pressure of silver oxide at 25° from the pressures at the higher temperatures.

In order to decide whether we are dealing with the same phase of silver oxide at high and low temperatures, we will determine from a change of the decomposition pressure between 302° and 445° the heat of decomposition of the silver oxide occurring at these temperatures, and compare this value with the one obtained at ordinary temperatures by other methods.

The heat of decomposition may be found from the change of pressure with the temperature, by the aid of the equation of van't Hoff, which in this case has the form,

$$\frac{dlnp}{dT} = \frac{Q}{RT^2} \qquad (1)$$

where p is the decomposition pressure, T the absolute temperature, R the gas constant, and Q the heat absorbed when 2-gram molecules of silver oxide decompose according to the equation,

$$2\ Ag_2O = 4\ Ag + O_2.$$

This equation of van't Hoff is not strictly exact, but is derived with the aid of two assumptions from the equation of Clausius,

$$\frac{dp}{dT} = \frac{Q}{T\ (v - V)} \qquad (2)$$

where v is the molecular volume of oxygen at the temperature T and the pressure p, V is the total change of volume of the solid system—that is, it is the volume of 2-gram molecules of silver oxide less that of 4-gram molecules of silver. Q represents the heat absorbed during the decomposition and may be replaced by the expression $U + p\ (v - V)$, where U is the increase in internal energy accompanying the decomposition, and $p\ (v - V)$ is the work done. In the integration of the van't Hoff equation U is usually regarded as constant, but since we are dealing with a pretty wide range of temperature we shall obtain a more accurate result by regarding it as a linear function of the temperature according to the equation

$$U = U_0 - C\ T \qquad (3)$$

Where C is the diminution in the heat capacity of the system during the decomposition of two gram-molecules of silver oxide. We may therefore write in place of the equation 2,

$$\frac{dp}{dT} = \frac{U_0 - CT + p\ (v - V)}{T\ (v - V).} \qquad (4)$$

In order to obtain equation 1 from equation 2 or 4, it is necessary to make two assumptions; first that the oxygen obeys the gas law, and second, that the volume V is negligible compared with v. Since the deviation of oxygen from the gas law is small at ordinary temperatures[7]

[7] Amagat (l. c.).

and is undoubtedly much smaller at the temperature of our experiments, the first assumption will not lead to any appreciable error. The second assumption would also be justifiable if it were not for the fact that in our experiments at 445° the pressure is so great, and consequently the volume v so small, that in comparison with it V is large enough to be considered.

Regarding V not as a negligible but as a constant quantity and assuming that $pv = RT$, I have succeeded in obtaining the following integrated form of equation 4,

$$ln\frac{p_1}{p_2} = -\frac{U_0}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) + \frac{V}{R}\left(\frac{p_1}{T_1} - \frac{p_2}{T_2}\right) + \frac{R-C}{R}\,ln\frac{T_1}{T_2}.$$ [1]

With the aid of this equation we are able to calculate from the decomposition pressures at any two temperatures the value of U_0. In order to do this we must know the values of V and C. The terms in which these quantities occur are comparatively unimportant ones in the equation and since both these quantities can be disregarded altogether without very seriously influencing the result, their approximate values will suffice.

From the tables of Landolt and Börnstein we find for the densities of silver and silver oxide 10.5 and 7.5, respectively. Calculating from these the molecular volumes we get approximately 20 cc. as the value of V.

The specific heat of silver oxide has not been determined, but the value of C may be found from the principle of the constancy of the atomic heat in solids. The heat capacity of silver is doubtless approximately the same in the oxide and in the metal. C, therefore, is the difference between the heat capacity of oxygen in the gaseous and solid states. The heat capacity at constant volume of 32 grams of oxygen in the gaseous state is 5 calories per degree. The heat capacity in the solid state is about 8.[2] Whence C equals 3.

We will determine the value of U_0 in calories and therefore use for R the value 2.0, except in the next to the last term of equation 5, in which, if the pressure is expressed in atmospheres and the volume in cubic centimeters, R must be expressed in corresponding units and given the value 83.

We have three sets of experimental values (*a*) $T = 445 + 273$, $p = 207$; (*b*) $T = 302 + 273, p = 20.5$; (*c*) $T = 325 + 273$, $p = 32$. These may be used in equation 5 in pairs. Using values (*a*) and (*b*) the first term of the equation, $ln\frac{p_1}{p_2}$ has the value 2.31; the third term,

[1] This equation may readily be verified by differentiating it. The resulting equation by suitable transposition and with the aid of the equation $pv. = RT$, can readily be shown to be identical with equation 4. Equation 5 may also be obtained by an independent method from equations 6 and 7 of my paper entitled "Law of Physico-Chemical Change." (*Zeit. Phys. Chem.* (1901) **38**, 205.)

[2] See, for example, Ostwald's Lehrbuch.

0.06; the fourth term, — 0.11. Combining these, we get for the second term the value 2.36, whence U_0 is 13,600 in small calories.

Using similarly values (*a*) and (*c*) we find for U_0 the same value, 13,600 calories.

From values (*b*) and (*c*) we find $U_0 = 13,300$. This value is to be given less credit on account of the nearness of the two temperatures used.

The remarkably good agreement between these values shows conclusively that we are dealing with the same reaction throughout the whole range of temperature from 302° to 445°. As final result we will take, $U_0 = 13,600$ small calories, or 136 K (Ostwald calories). From equation 3 the value of U at room temperatures is therefore between 127 K and 128 K. This is the increase of internal energy accompanying the decomposition of 2-gram molecules of silver oxide. The heat of formation of silver oxide at room temperatures, exclusive of external work, is therefore one-half this value or 64 K.

HEAT OF FORMATION OF SILVER OXIDE, OTHERWISE DETERMINED.

For the heat of formation of silver oxide we find in the literature only two determinations which are at all trustworthy. Thomsen[10] allowed copper to act upon silver nitrate and from the heat of this reaction, the heat of formation of copper nitrate, and the heat of neutralization of silver oxide, calculated the heat of formation of the latter to be 59 K (*a*). Berthelot[11] by a similar method obtained 70 K (*b*).[12]

In his experiments on the energetics of galvanic elements, Jahn[13] obtained the following equation:

$$Pb + 2\ AgNO_3, Aq = Pb\ (NO_3)_2, Aq + 2\ Ag + 509\ K.$$

Let us combine this equation with the following:

$$PbO + 2\ HNO_3, Aq = Pb\,(NO_3)_2, Aq + H_2O + 178\ K\ \text{(Thomsen)}$$

$$Pb + O = PbO + 503\ K\ \text{(Thomsen)}$$

$$Ag_2O + 2\ HNO_3, Aq = H_2O + 2\ AgNO_3, Aq + 104\ K^{14}\ \text{(Berthelot)}$$

Whence

$$2\ Ag + O = Ag_2O + 68\ K\ (c)$$

[10] Not having Thomsen's book at hand, the values here attributed to him are taken from Ostwald's Lehrbuch.

[11] *Ann. de Chim.* (1878) **15**, 186.

[12] All these values are for the "total" heats of reaction. I have chosen to use these values, which include the external work, since they are the ones commonly given in the literature. The reduction to the simple "internal" heat of reaction will be made later.

[13] *Wied. Ann.* (1886) **28**, 493.

[14] This value will be discussed presently.

Recently Jouniaux[15] has determined the conditions of equilibrium at several temperatures between silver, hydrochloric acid gas, silver chloride and hydrogen, and has thus obtained the thermo-chemical equation,

$$HCl\ (gas) + Ag = AgCl + H + 71\ K^{16}$$

Let us combine with this equation the following equations of Berthelot:

$$HCl\ (gas) + Aq = \overset{+}{H} + \overset{-}{Cl} + 174\ K.$$
$$\overset{+}{Ag} + \overset{-}{Cl} = AgCl + Aq + 157\ K.$$
$$\tfrac{1}{2}\ Ag_2O + \overset{+}{H} = \overset{+}{Ag} + \tfrac{1}{2}\ H_2O + 52\ K.$$
$$H + \tfrac{1}{2}\ O = \tfrac{1}{2}\ H_2O + 345\ K.$$

Whence

$$2\ Ag + O = 2\ Ag_2\ O + 66\ K\ (d).$$

I have measured the E. M. F. of the following cell,[17]

$$Hg \mid HgCl \mid KCl\tfrac{N}{10} \mid KNO_3\tfrac{N}{10} \mid AgNO_3\tfrac{N}{10} \mid Ag$$

and found it to be 0.429 — 0.00120t, volts, where t is the temperature Centigrade. From these figures the Helmholtz equation gives $Hg + \overset{-}{Cl} + Ag = Ag + HgCl + Aq + 175\ K$. With this equation we will combine the following:

$$\tfrac{1}{2}\ Ag_2O + \overset{+}{H} = \overset{+}{Ag} + \tfrac{1}{2}H_2O + 52\ K^{18}$$
$$HgCl + Cl = HgCl_2 + 219\ K^{19}$$
$$Hg + 2\ Cl = HgCl_2 + 533\ K^{20}$$
$$Cl + \tfrac{1}{2}\ H_2O + Aq = \overset{-}{Cl} + \overset{+}{H} + \tfrac{1}{2}O + 50\ K.^{21}$$

Hence

$$2\ Ag + O = Ag_2O + 74\ K\ (e).$$

[15] *C. R.* (1903) **132**, 1270.

[16] I have added 3K to the value given by Jouniaux in order to obtain the "total" heat of reaction.

[17] The details will be given in the following paper.

[18] Thomsen gives 54 K, but Berthelot found 52 K by the neutralization of silver oxide by nitric acid (*Ann. de Chim.* (5) (1875) **4**, 188), and also precipitation with alkalies (*ibid* (5) (1375) **4**, 503) and has recently verified the value by new experiments (*C. R.* (1901) **133**, 555).

[19] This value was obtained by Thomsen from the direct action of chlorine upon calomel. It is in all probability correct, as it has not been called into question in the critical work on mercury salts of Nernst (*Zeit. Phys. Chem.* (1888) **2**, 22) and Varet (*C. R.* (1893) **120**, 620 and 921).

[20] This value was obtained independently by both Nernst and Varet (l. c.).

[21] Thomsen's value is 50.5 K (*Pogg. Ann.* (1873) **148**, 177). Berthelot's is 48 K (*C. R.* (1889) **109**, 546 and 590). Thomsen's value is probably the more accurate.

We have thus found five independent values of the heat of formation of silver oxide, varying from 59 K to 74 K. These values all include the external work. In order to obtain the mere change of internal energy, 3 K must be subtracted from each of the above numbers. We have then,

(*a*)	56
(*b*)	67
(*c*)	65
(*d*)	63
(*e*)	71

The average of these values is 64 K, which happens to be precisely the value which we obtained in the preceding section. This very satisfactory agreement proves conclusively that the silver oxide which exists between 302° and 445° is, from a thermo-dynamic point of view, identical with that which exists at ordinary temperature.

DECOMPOSITION PRESSURE OF SILVER OXIDE AT 25°.

We are now able to calculate from equation 5 the equilibrium pressure between silver oxide, silver, and oxygen at 25°. As data we will take 20.5 atmospheres as the pressure at 302°; 64 K as the heat of formation of silver oxide at 25°; and for C the value 3, as before. The third term of the equation containing V may be here neglected. We thus obtain $p_{25} = 4.9 \cdot 10^{-4}$ atmospheres.

When we consider the question of how much this value is affected by possible errors in the data used, we find that errors in p_{302} and in C have much less influence than an error in U_{25}. An error of 1 K in the latter would cause an error of nearly 20 per cent in p_{25}. In the following paper I shall show that from this value of the decomposition pressure of silver oxide at 25° it is possible to calculate the electrolytic potential of oxygen and that the value thus obtained differs very considerably from the one at present accepted.

SUMMARY.

The equilibrium pressures in the system, silver oxide, silver and oxygen, at the temperatures 302°, 325°, and 445° are shown to be respectively 20.5, 32 and 207 atmospheres.

It is proved that no silver suboxide was present in any of the experiments and that probably it is incapable of existence at these temperatures.

From the change of equilibrium pressure with the temperature, the heat of formation of silver oxide is found to be 64 K (Ostwald calories).

The mean of five determinations of this quantity based on calorimetric data is likewise 64 K.

The decomposition pressure of silver oxide at 25° is calculated to be about $4.0 \cdot 10^{-4}$ atmospheres.

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PHILIPPINE FIBERS AND FIBROUS SUBSTANCES: THEIR SUITABILITY FOR PAPER MAKING.

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RAW MATERIALS FOR PAPER MAKING.

Introduction.—From the earliest Egyptian papyrus to the paper of to-day, the predominant characteristic of this material is that it consists of the enduring portions of vegetable growth known as cellulose, although animal and mineral fibers such as wool, silk, and asbestos are occasionally employed. The art of modern paper making consists of uniting or felting together any fibrous material so as to form a continuous sheet. Linen or cotton rags are no longer exclusively employed; indeed, these substances at present constitute but a small fraction of the raw material of the paper-making industry. Any vegetable matter possessing sufficient fibrous structure can be utilized.[1]

Notwithstanding the great variety of available cheap materials, rags of various kinds continued to form the chief substances for paper making both in Europe and America, until the middle of the nineteenth century, at which time they ceased to be obtainable in sufficient quantities to supply the demand and paper makers began to search elsewhere for a cheaper and more inexhaustible material for their rapidly growing industry. In 1854 wood-pulp was first used in the United States, and

[1] "In order to give some idea of the variety of materials from which paper can be and has been prepared, we may cite a book published in 1765 at Regensburg, Germany, by Jacob Schaeffer, the paper of which was made from about sixty different sources, among which the following are curious and interesting examples: Sawdust, hop vines, hornets' nests, peat, straw, cabbage stumps, moss, and thistle stalks." Thorpe: *Dictionary Applied Chemistry*, 3, 105.

three years later Mr. Thomas Routledge introduced esparto grass into England. The simultaneous introduction of wood and grass furnished the first important sources of raw material for paper making and provided the first evidence that perennial grasses are suitable for paper stock.[3]

It is interesting to note the direction which search for suitable paper material was taking when the adaptability of wood for this purpose was first discovered and also to predict the lines of future inquiry when wood no longer meets the demand. When, in 1861, all import duties in Great Britain were repealed, the resulting establishment of a vast number of weekly and daily papers and journals created so great a demand for paper and paper pulp that manufacturers were forced to supplement the imported Spanish and North African esparto grass with the cereal straws, but even these proved insufficient to meet the requirements and, as the prosperity of English paper mills appeared to be at stake, the demand seemed justified that the Indian bamboo forests be thrown open to private enterprise; accordingly, Mr. Thomas Routledge, a prominent paper manufacturer of Sunderland to whom the introduction of esparto is due, sent investigators to India to study the problem in that country. However, about this time the manufacture of paper stock from spruce timber had been developed on the Continent, particularly in Germany and Sweden, and supplies of this new material from those countries brought the much-needed relief; nevertheless, experiments were carried far enough to demonstrate that bamboo fiber is much superior to spruce for paper stock and there seems but little doubt that the bamboo-paper question eventually will be reopened.

In America the evolution of raw material for paper making followed somewhat different lines. The transition from rags to wood was direct and was later followed by the use of straw in those regions far removed from the spruce forests. No recourse to perennial grasses or bamboo has thus far been necessary.

For half a century wood-pulp has met the rapidly increasing demand for paper stock. However, we are now confronted with the fact that the supply of this material will soon be exhausted, so that we are afforded a curious example of the manner in which the development of an industry sometimes brings one back to the conditions of the beginning, although the new point reached, owing to the persistence of scientific inquiry which was undertaken regardless of an utter lack of apparent practical benefit, is on a much higher plane.

Until wood-pulp had been proven to be suitable for paper stock, the world's supply of fibrous material was divided between the textile and paper industries, one being complementary to the other. Such vegetable

[3] As fibers of cotton and flax in the form of cotton and linen rags have already undergone purification and have been subjected to processes of manufacture, they can not, strictly speaking, be considered as raw materials.

fibers as cotton, hemp, jute, flax, and abacá are eminently suited for the manufacture of paper, but their primary value for textiles and cordage excluded their use in the raw state for paper and therefore, the paper makers obtained their material largely from the refuse of these industries. Good cotton and linen rags have become a luxury in the paper-making world. They are only indulged in now for making the best class of stationery, and by fortunate coincidence, this is about the only use to which they can be put. At first glance, wood might be considered too valuable for other purposes, but fortunately, those varieties which find most favor for the making of paper pulp are considered rather worthless for the many other uses for which wood is usually employed; furthermore, the demands of the spinner and cordage maker need not be considered. Twenty or twenty-five years ago statements "that there is not the slightest ground for believing the supply of this raw material would ever fail" were common in regard to wood as a material for paper making. The marvelous growth in the paper industry of the last two decades was not then foreseen nor were the many other uses for wood-pulp, which modern advances in the industrial world have brought about, taken into account.

P. H. Clutterbuck, referring to the numberless uses of wood-pulp, writes:[3]

> Printing paper alone eats an enormous hole in our natural forests yearly and the future requirements can only be conjectured. The huge procession of railway cars all over the country run, to some extent, on paper wheels; carpenters are beginning to use boards of paper, handsomely veined, requiring no planing, twice as durable as the wooden variety and costing only half the money. The builder is introducing paper bricks, showily enameled, which will not burn and possess many advantages over those of clay. The shipbuilder introduces masts and spars of the same substance, which is likewise used for telegraph and telephone poles and flagstaffs. These are not fanciful experiments but serious business procedures, justified by superior durability of the articles so produced. This same quality is claimed for the paper horseshoe recently invented and so extensively used.

Already, paper manufacturers in the United States are looking for new sources of supply for raw material. A recent report of the United States Department of Agriculture[4] recommends that investigations be made on the suitability of new raw materials for paper and paper pulp.

"Our well-known pulp woods are being used up faster than they are growing, and as a consequence the demand for new material has led to efforts to utilize many waste products among which bagasse or sugar-cane refuse, cornstalks, southern pine waste, rice straw, and hemp stalks present exceeding promising fields."

The United States Government recently has established a laboratory at Washington for investigations along these lines, and this fact emphasizes the importance which the question is assuming.

[3] P. H. Cutterbuck: *Indian Forester* (1899), 25, 231.

[4] U. S. Department Agrl.: *The report of the Chemist* (1904).

PULP AND PAPER MAKING.

During the nineteenth century there were remarkable changes and improvements in the methods employed for converting paper stock into paper pulp and paper. These advances have been due to two causes—one, the revolution in the nature and supply of the raw material itself, and the other, the increased demand for the finished product. The method of preparation of paper pulp or half-stuff has thus far largely been dependent upon the nature of the material treated, whereas the making of the pulp into finished paper in sufficient quantities to meet the marvelous growth of the industry has caused the laborious hand process to be superseded by the huge automatic machines of the present time. However, the principles involved in the making of paper remain unaltered, regardless of whether the material is removed from a vat with a small hand sieve and turned out a single sheet at a time or is allowed to flow on to an endless wire cloth web under heavy rolls and over the steam-heated drying cylinders of a Fourdrinier machine. Generally speaking, the purpose is just the opposite of that which obtains in the isolation of fibers for the textile and cordage industries; instead of so treating the fibrous substance as to preserve the fiber bundles or filaments in their greatest length, it is necessary, by some mechanical or chemical means, to convert them into the individual fibers or cells of which the filaments are composed.

There are five distinct steps in the preparation of paper pulp from any vegetable material. Two of these are entirely mechanical, whereas the remainder are of a distinctly chemical nature. Arranged in their order of procedure, they are:

1. *Cleaning.*—A purely mechanical process which consists in removing all foreign matter such as sand, dirt, weeds, chaff, etc., either by hand or machinery.

2. *Boiling or digesting.*—This results in eliminating the soluble plant constituents and incrusting matter by chemical means.

3. *Bleaching* consists in further chemically purifying the resistant cellulose by removing adhering coloring matter.

4. *Beating or refining.*—This procedure mechanically disintegrates the pulpy mass of fibers into fragments of requisite length.

5. *Loading, sizing, and coloring* so modify the bleached and beaten pulp by the addition of mineral or animal substances, that a nonporous, resistant surface of the required shade is given to the finished product.

BOILING OR DIGESTING.

At the present time there are two main groups of processes in general use for the isolation of paper cellulose, namely, the alkaline and acid treatments. The first and older method depends upon the action of solutions of caustic soda, soda ash, caustic lime, or mixtures of these

chemicals, under varying conditions of strength of solution, pressure, and duration of digestion.[5] Therefore it is evident that in valuing an unknown material for use as paper stock these differences need carefully to be considered.

The second method for the resolution of raw fibers is of comparatively recent origin and consists in cooking them under strong pressure with sulphurous acid, either free or combined with soda, lime, or magnesia in the form of the bisulphites of these bases. The development of this process has been slow, owing to the many mechanical difficulties involved, the strong chemicals employed attacking and soon rendering the digesters worthless. However, within recent years resistant digester linings have been invented and now this process is established as the leading method for the preparation of chemical wood-pulp. Comparatively little has been done to show its adaptability to materials other than wood, but the process has so many features of superiority over the alkaline one that its application in the treatment of various other materials will be considered in a subsequent paper when work on the value of Philippine woods for paper stock will be reviewed.[6]

Bleaching of paper pulp.—All fibers do not act alike with bleaching agents. Jute, for example, does not bleach white by any known method which does not also seriously injure the fiber, while other substances require varying proportions of bleach liquor and special conditions of treatment to secure satisfactory results. Therefore, it is important to subject new, raw materials to quantitative bleach operations in order to determine the right method of procedure and the amount of bleaching powder required. The loading, sizing and coloring of paper are not materially affected by the source from which the pulp is derived and need only concern us here with respect to the kinds of chemicals in general use for these purposes and the possibilities of obtaining them in the local market. The question of chemicals for this and the other steps in the process of pulp manufacture is an important one and will thoroughly be discussed in a later number of this journal.

VEGETABLE FIBERS.

Botanical classification of fiber-producing plants.—Fiber plants are included in two great divisions of the vegetable kingdom—i. e., Dicotyle-

[5] Esparto grass is invariably subjected to the alkaline method of treatment, but the pressures carried vary from 5 to 50 pounds, the time of digestion from one to six and one-half hours and the strength of the caustic liquor from 10 to 20 per cent, calculated upon the gross weight of the material.

[6] "On account of the considerable proportion of silica present in straw, it has generally been assumed that this material would not easily lend itself to treatment by the sulphite process. Practical experience has, however, shown that this is not the case, and this process has recently been applied to the preparation of straw pulp with excellent results." Griffin & Little: *Chem. of Paper Making*, 161.

dous and Monocotyledons. The most evident characteristic of these two great divisions of plants is found in the arrangement of the leaf veins. Dicotyledonous plants are characterized by netted-veined leaves, whereas the monocotyledonous ones may usually be identified by leaves with parallel veins. The chief fiber-yielding families of the first division are:

Linaceæ; Flax family; example, flax.

Malvaceæ; example, cotton.

Tiliaceæ; example, jute.

Urticaceæ or nettle family; example, hemp.

Moraceæ; example, mulberry.

Of the second division the *Gramineæ* or Grass family, *Liliaceæ*, *Palmæ*, or Palm family, and *Musaceæ* or Plantain family are the more important orders of fiber producers. While a number of netted-veined, fibrous plants such as ramie and jute are distinctly tropical in habitat, only a few unimportant species are found in the Philippines, and hence it appears that families of Monocotyledons are the only ones represented by plants of commercial importance in these Islands.

Structural classification.—With the exception of fibers like cotton, kapok, etc., which are unicellular seed hairs and termed *surface fibers*, practically all fibers may structurally be classified according to the two main groups of families from which they are derived. The dicotyledonous plants produce the so-called *bast fibers*, contained in the inner fibrous bark of stems and twigs, while on the other hand the commercial fibers of monocotyledonous plants are generally found distributed throughout the entire stem, where they form the framework which gives rigidity and toughness to the plant structure, and hence they are termed *structural fibers*. Such fibers occur in the sheathing leafstalks of plantains, in the fleshy leaves of maguey and pineapple, and in the leaves and stalks of palms and grasses.

Economic classification.—A descriptive catalogue of the useful fiber plants of the world by C. R. Dodge[7] enumerates over one thousand species, the more important of which are fully described and treated from the botanical, structural, and industrial standpoints. His classification of fiber plants based on their uses is both so simple and natural that we incorporate its main features, at the same time drawing on local fiber plants for illustrating the numerous divisions of the scheme:

A. *Spinning fibers.*
   1. Fabric fibers; pineapple, abacá, ramie, etc.
   2. Netting fibers; palms, rattan, bamboo.
   3. Cordage fibers; abacá, maguey, bamboo, rattan.

B. *Tie material* (rough twisted). Palms, rattans, bamboos, grasses.

C. *Natural textiles.* Fibrous sheaths of palms.

D. *Brush fibers.* Palm fibers, bamboo.

[7] *Report No.* 9, U. S. Department of Agriculture.

E. *Plaiting and rough weaving fibers.*

1. Articles for attire; hats, sandals, etc. Abacá, palms, bamboo.
2. Mats and mattings; also thatch materials. Grasses, bamboos, palms, etc.

F. *Forms of filling.* Kapok, straw, grasses.

G. *Paper material.*

1. Textile papers. All waste from A, including old rope.
2. Bast papers.
3. Palm papers. From the fibrous material of palms and similar monocotyledonous plants, including rattans.
4. Bamboo and grass papers. This includes all material from graminaceous plants, including bamboos, cereal straws, and true grasses.
5. Wood pulp papers. Philippine soft woods, Lauan (*Shorea*), Cupang (*Parkia*), *Grewia*, etc.

It will be seen from this scheme of classification and from the native plants selected to exemplify each division of use, first, that a comparatively small number of plants supply fiber for all the present requirements; for instance, plantains, grasses, bamboo, rattan, and palm fiber are made into fabrics, fish nets, hats, baskets, mats, twine, rope, thatch, brushes, and brooms; second, that those plants which find such general use are without exception monocotyledons and their fibers are of the class termed *structural;* third, that with the exception of maguey and pineapple they are either plantains, grasses, or palms; fourth, that, leaving out of consideration native woods as a possible source of paper stock, the available supply of material for any future paper industry in the Philippines must come from one or more of these three sources.[8]

Chemical examination of raw material.—In making a study of an unknown, fibrous substance with the view of determining its possible utilization for paper stock, the three most important points to be considered are, first, its cellulose content, namely, the percentage of pulp which a given raw material will yield; second, the ease with which it responds to the ordinary cooking and bleaching processes necessary for its conversion into paper pulp; and third, the physical characteristics of the individual fibers—that is, the nature of the fiber bundles and the dimensions of the ultimate fibers of which they are composed. If the above information is favorable—namely, if the raw material is relatively high in resistant cellulose; if it is easily reduced and readily bleached, taking into account the strength of liquors required; if the ultimate fibers are found to be long and thin, with tapering, curly ends—then the question of distribution, availability, and cost of collection of that raw material is well worth careful consideration. While a quantitative determination of cellulose on a 5-gram sample of material affords some basis for estimating the amount of paper pulp it will produce, the results thus

[8] Maguey culture is rapidly increasing in northern Luzon, and the waste from the stripping of the plant may become an important factor in paper making.

obtained [9] are invariably much higher than those recorded in the factory, where much loss of fiber due to the practical, time-saving methods in vogue, is inevitable. Furthermore, many raw materials—abacá waste, for instance—are of such a nature as to render the taking of a small, representative sample practically impossible.

The method adopted in the laboratory for determining the percentage of cellulose or paper pulp is that used on a larger scale in the factory—i. e., severe alkaline digestion at elevated temperatures. It is also true that the ease with which the cellulose may be obtained can only properly be established by direct experimentation under factory conditions. To meet these, we had constructed a model digester of sufficient capacity to accommodate several pounds of material and so arranged that it could be converted for use with alkali or sulphite liquors, with the necessary steam and temperature gauges for carrying out digestions at elevated pressures.

Proceeding with the chemical examination of a given fibrous substance, there are other questions to be determined before the value of a raw material as a source of paper stock is fully expressed. Besides knowing the proportion of paper cellulose contained in the material to be considered and the ease with which it may be isolated, it is necessary to form some idea of its chemical nature—i. e., its general composition and behavior with hydrolytic and oxidizing agents—for on such knowledge our ideas of the purity and stability of the manufactured product are based.

PHILIPPINE FIBERS.

In looking over the field for suitable material for purposes of study, our attention was first directed to abacá, or Manila hemp, as it sometimes is termed. This plant is a species of wild banana of which there are several indigenous to, or introduced into, the Philippine Islands, and, as is well known, so-called Manila paper has long been made from worn-out ropes of this fiber. Its superior quality for the manufacture of special kinds of paper where strength is the desideratum is well demonstrated.

According to the Twelfth Report of the United States Census, American paper manufacturers purchased approximately 100,000 tons of so-called Manila stock for use in their mills; the approximate cost was $2,500,000, or $25 per ton. This stock was made up of old, worn-out rope, gunny sacks, waste thread, and binder twine, and it includes jute

[9]

	Laboratory method. (Yield of dry cellulose on dry raw material.)	*Paper maker's method.* (Yield of air-dry pulp on air-dry raw material.)
	Per cent.	Per cent.
Esparto	50–55	43–47
Straw	50–55	33–37
Wood	50–50	35–43

Cross and Bevan: *Cellulose*, 284.

butts and waste. Rags, which the world over are considered to be the finest material for paper making, do not command a much greater price. In the same census year (1900) 234,000 tons of rags valued at $6,600,000, equal to about $28 per ton, were used. Statistics also show an annual output of 90,000 tons of Manila wrapping paper valued at $66 per ton and 204,000 tons of "bogus" Manila paper made from wood, rated at $9,150,000, or $15 per ton. A large proportion of the so-called Manila wrappers, paper, and envelopes contain mechanical or ground wood, which causes such articles to deteriorate and to turn brown with age. G. E. Marshall, of Turners Falls, Massachusetts, has patented a process for giving the characteristic color of Manila stock to wood-pulp and such attempts at imitation and adulteration show very conclusively the repute in which real abacá stock is held.

Abacá waste.—It has been observed for a long time that much fiber is lost in the ordinary hand processes for isolating the cordage fiber from this plant, and, in 1887, some of this waste from abacá was sent to Messrs. Gonzales Sons, of Barcelona, Spain, who reported as follows:

Observations made in the course of fabrication permit us to affirm roundly that abacá waste as raw material for the manufacture of paper is not only utilizable but surpasses esparto and hemp, and, in its treatment for conversion into paper, excels rags and other material known in the industry.

In 1888, Sr. D. José Jordana published a small pamphlet entitled "El Abacá," containing a specimen sheet of paper made from abacá waste and also a section devoted to the application of this material for the manufacture of paper, from which the following brief extract is taken:

As with all vegetable filaments, excellent paper can be made with abacá, even though this use is not made of it on account of its greater value as a textile. The waste which remains after removing the pulpy envelope could also be used for this manufacture. This envelope is left in the fields in its entirety, where it serves only as fertilizer for the plants; and on account of the imperfections of the method of extraction the quantity of this is so great that it exceeds 75 per cent of the total of the solid part harvested. In any case, industry and commerce should give attention to this matter. The abundance, cheapness, and excellence of a raw material are guaranties of all industrial enterprises, and this condition is found to-day in abacá waste.

In 1904, a quantity of this waste material was sent to the Everett Pulp and Paper Company, of Everett, Washington, U. S. A., for further opinion as to its adaptability for paper manufacture. Through the courtesy of the Bureau of Agriculture of the Philippine Islands we give the chemist's report:

The waste as received was very dirty, and could not be used in the way we now treat our wood, but would have to be passed through a duster or "Devil," such as is used for very dirty rags, oakum, rope, etc. It may be possible in collecting this material to avoid a large part of this dirt.

The waste was submitted to the same process as the wood now being used, except the caustic-soda solution was one-half the strength, the time of digesting four and one-half hours, the pressure 100 pounds, and 325° Fahrenheit temperature.

The cooking was done by indirect steam—that is, the heat was produced by a steam coil in the digester, and not direct steam as we use it.

The fiber reduces very easily, and, after being washed in hot water, bleaches to color of sample, with the same amount of bleaching powder as required by the wood pulp.

The loss in the process from the dry, crude material to the dry, bleached pulp is 64 per cent, or, the crude material will produce 36 per cent by weight of fiber ready for the beater.

Under the microscope the fibers are long, slender, and smooth, tapering to a point at the ends; some have small hairs on the sides.

The bleached fiber contained 6.8 per cent ash, while wood fiber has less than 2 per cent and in most instances when well cooked has only 0.3 per cent.

This however, does not detract from the value as a paper-making material.

Owing to the small amount of material received, it has been impossible for me to try any experiments along the line of obtaining the same results by the use of cheaper chemicals. It might be possible to do the work with a solution of lime, the same as straw is treated.

In my opinion this abacá waste can be reduced to a fine, silky fiber, suitable for the best of paper, making a sheet equal to the best linen, or without being bleached, made into a light-gray wrapping paper, which would excel in strength any paper on the market.

In the meantime, the Bureau of Agriculture of the Philippine Islands conducted a series of experiments in stripping abacá by the customary hand process for the purpose of determining the relative amounts of dry cordage fiber saved and dry fibrous waste lost.[10]

Weight.	San Ramon, Mindanao.	Paete, Laguna.
Weight of green stalk .... kilos..	9.525	15.876
Weight of green fiber .... do.....	.4536	.680
Weight of dry fiber .... do....	.163	.2268
Weight of green fibrous waste .... do....	1.814	2.494
Weight of dry fibrous waste .... do....	.1769	.199
Percentage of dry waste in stalk	1.85	1.25
Percentage of dry fiber in stalk	1.7	1.43

The importance of the above data because they seriously influence our judgment of the supply of this waste material, justifies their repetition, particularly as we also subsequently desired to learn the effect of machine stripping on stalks under the same conditions. The following table gives a comparison between the hand and machine stripping processes; the stalks used were from Paete, Laguna, and had been harvested about two

[10] *Report* of H. T. Edwards, Fiber Expert, Bureau of Agriculture, Manila, P. I. (1904).

weeks before the tests were made. The results are averaged from ten stalks:

Weight.	Hand stripped.	Machine stripped.
Green stalk .... kilos..	15.876	13.984
Green fiber .... do....	.317	.199
Dry fiber .... do....	.159	.1147
Green fibrous waste .... do....	.273	....
Dry fibrous waste .... do....	.199	.635
Percentage of dry waste per stalk	1.25	4.54
Percentage of dry fiber per stalk	1.00	.821

Explanation of results.—In order to explain the data recorded in the above table it is necessary to describe the manner in which the stalks were prepared for the two methods of treatment. For the hand stripping, about one foot of each end of the stalks was chopped off to facilitate the removal of the petioles, and three or four of the outer ones, which were bruised and discolored, were also discarded. Then, two or more ribbon-like strips were peeled from the entire length of the outer surface of each layer of the remaining portion of the stalk. Only these thin, fibrous ribbons were subjected to the stripping process. All the cellular portions of each petiole, together with the outer sheathes and the cut ends, were discarded. These constitute a class of waste distinct from that which results from the stripping operation itself. For reasons which will appear later, this waste is designated as *semifibrous* in distinction from the genuine *fibrous* material collected under the stripping knife.

In the case of the machine-stripped fiber, the stalks were prepared by first removing the ends and outer petioles, as in the method mentioned above, but the remainder was cut lengthwise into sections of the requisite width and then fed directly into the machine, without any attempt being made at the separation of the fibrous from the cellular portions. Hence, the 635 grams of dry waste which remained represented the quantity produced by the actual stripping process and must directly be compared with the 199 grams of dry, fibrous waste obtained by the other method. The machine removed so much water from the waste that its weight in a green state would have afforded no value for comparison; therefore it was not determined.[11]

The machine employed for this test was still in an experimental stage, hence the results obtained do not afford reliable data as to the possible

[11] Concerning the percentage of mercantile fiber produced by the two different methods of stripping, it will be observed that only 114 grams per stalk were obtained by machine stripping, as compared with 159 grams per stalk by hand stripping. However, it is only fair to state that the machine-stripped fiber was of better quality, being more polished and drier, as the comparative weights of green and dry fiber show.

effect of machine stripping on the future supply of abacá fiber and waste, but it was the only opportunity which has thus far presented itself for obtaining even approximate information in regard to this question. This machine waste contains a large amount of fiber of value to the paper maker and the quantity of waste demonstrates the fact that, whereas the outer surface of the leaf petioles undoubtedly contains the strongest fibers which are so valuable to the cordage maker, the cellular portion of the petioles also contains much fiber which is entirely lost by the hand-stripping process.

Semi-fibrous waste.—No determination of the percentage of the semi-fibrous waste, above referred to, was made at the time the stripping experiments were conducted. Later, an approximate determination of the amount produced by a given stalk was arrived at in the following manner: A complete stalk of abacá, weighing 15.876 kilos, was chopped into small pieces and subjected to hydraulic pressure. The dried residue weighed 1.592 kilos, so that the original stalks contained 10 per cent of vegetable matter and 90 per cent of water, calculated on the gross weight. In the case of the hand-stripped stalks only 2¼ per cent of these solids is accounted for. This loss of nearly 80 per cent, or four-fifths of the total solids of the stalk, caused us to collect and dry some of this waste material for a determination of its cellulose content. These and all subsequent digestion experiments were conducted on 1-kilo lots of dry material.

Experiment No. 1.—Strength of the caustic soda liquors, 2 per cent; 15 per cent of caustic soda calculated on dry weight of material treated; pressure, 6–8 atmospheres; time, 8 hours; yield, 33 per cent; digestion, incomplete; residue, brown and coarse and only fit for cheap, colored wrappings.

Experiment No. 2.—Strength of liquor, 3¼ per cent; 25 per cent caustic soda calculated on dry weight of material; pressure, 6–8 atmospheres; time, 8 hours; yield, 20.5 per cent; apparently well digested; light-gray residue.

Experiment No. 3.—Strength of liquor, 5 per cent; 45.5 per cent caustic soda calculated on dry weight of material; other conditions same as above; yield, 20 per cent.

From the above results we see that this waste is semi-fibrous. The time required, the pressure, and the strength of the caustic soda necessary to remove the cellulose portions of this waste will undoubtedly prohibit its use. No further experimentation with this material was made. For the manufacture of wrapping paper, where color is not so important as is strength and cheapness, the waste could be lime-boiled under pressure, and but partially bleached, as is done with jute.

Fibrous waste.—The waste material, which results from the present method of extraction of the fiber for cordage and textile purposes, consists essentially of broken, tangled, but clean, fibers mixed with strips or bands of fiber aggregates, from which the cellular and incrusting matter has not entirely been removed. Its cellulose content varies, being of course dependent upon the amount of these soluble constituents which

are present. The readiness with which it is reduced to the condition of paper pulp, the kind and strength of chemicals required, and the time necessary for proper digestion likewise depend on this same factor. The fibrous waste was prepared for experimentation in much the same manner as old rope or rags are treated—namely, it was cut by hand into short pieces, thoroughly cleaned from all adhering sand and dirt and digested with caustic-soda liquor at elevated temperatures. The residues were drained and washed with hot water until they were free from alkali and the washings were colorless; they were then dried to constant weight in a water-jacketed air bath.

Experiment No. 1.—Strength of liquor, 2 per cent; 17 per cent caustic soda calculated on weight of material digested; pressure, 6–8 atmospheres; time, 4½ hours; yield, 30.65 per cent; residue, brown-gray in color, not entirely free from cellular matter.

Experiment No. 2.—Strength of liquor, 3¾ per cent; 25 per cent caustic soda calculated on weight of material; pressure, 6–7 atmospheres; time, 4½ hours; yield, 32.84 per cent; well-cooked pulp of a light-gray color.

Experiment No. 3.—Strength of liquor, 3¾ per cent; 30 per cent caustic soda calculated on weight of material; pressure, 6–7 atmospheres; time, 5 hours; yield, 31.7 per cent.

Experiment No. 4.—Strength of liquor, 4⅜ per cent; 40 per cent caustic soda; other conditions same as No. 3; yield, 31.64 per cent.

Experiment No. 5.—Five per cent liquor; 45½ per cent caustic soda; other conditions same as above; yield, 31.51 per cent.

The waste employed in the above experiments was obtained from a machine-stripping process. Reference to the table on page 443 shows the relatively high percentage of dry machine waste as compared with the hand-stripped article. This explains the low cellulose content obtained. The experiments bring out the following points:

First. Two per cent liquor is insufficient with the pressure and time employed.

Second. Three and three-fourths per cent liquor is as efficient as the stronger concentrations.

Third. The gradual but constant lowering of the yield in experiments 3, 4, and 5 is due to the fact that the stronger liquors attack the cellulose, but pressures of 6–7 atmospheres may safely be employed because, under these conditions, the solvent action is very slight.

The conditions of time, pressure, and strength of caustic soda which give the best results are approximately one-half of those employed in the manufacture of wood pulp by the soda process.

A second series of five digestions was made on abacá waste resulting from the hand process of extracting the fiber. The preliminary preparation of the material was the same as in the preceding experiments. In each case a 3¾ per cent caustic soda solution was employed, 25 per cent caustic calculated on the weight of the material digested. The pressure was held for five hours at 6 atmospheres as a minimum and 7 atmospheres as a maximum. The yields were 38.89, 41.69, 42.59, 42.66, and 42.14

per cent, respectively. The resulting pulps were of a light-gray color and devoid of the harsh feel which comes from insufficient cooking.

For purposes of comparison, two specimens of old Manila rope, representing the extremes of quality of such material for paper manufacture, were examined.

Preliminary treatment.—The pieces of rope were cut down and thoroughly cleaned and dusted as is the practice in mills where such material is used.

Specimen No. 1.—A good grade of 1-inch rope but badly frayed and worn; lost 21 per cent in cleaning.

Experiment No. 1.—Strength of liquor, 3½ per cent; 25 per cent caustic soda calculated on weight of material digested; boiled 5 hours at 6–8 atmospheres; yield, 61 per cent, equal to 48.7 per cent calculated on the original weight of rope.

Specimen No. 2.—A very dirty one-half inch rope of native manufacture, containing some unstripped strands of abacá; lost 21.82 per cent in cleaning.

Experiment No. 2.—All conditions of digestion same as above; yield, 43.89 per cent, equal to 34.31 per cent pulp, calculated on original weight of the rope.

Depletion of the land by removing abacá waste.—It is a fundamental principle of modern agriculture that all straw grown on a farm should be returned to the soil, and this applies to such by-products as cornstalks and sugar cane as well. The following analysis shows the comparatively low fertilizing value of abacá waste, so that its removal from the land in a dry state would not effect any appreciable loss of plant foods:

	Per cent.
Total nitrogen	0.52
Total phosphoric acid (P_2O_5)	0.046
Potash as K_2O	0.661
Lime (CaO)	0.238

It was shown on page 444 that 90 per cent of the green weight of an abacá stalk is represented by juice. This, on evaporation, was found to contain 2.62 per cent of solids or 275 grams from 15.876 kilos of stalk. A fertilizer analysis of this solid matter, obtained by evaporating the expressed liquid, was made by Mariano Vivencio, of this Bureau:

	Per cent.
Total nitrogen	0.40
Total phosphoric acid (P_2O_5)	1.86
Potash as K_2O	30.56

The presence of nearly 1 per cent of available potash in the juice is noteworthy because it shows the inorganic constituent needed by the growing plant and indicates the proper treatment for depleted abacá lands. Good wood ashes, the cheapest and best of potash fertilizers, contain on an average only 5 per cent of available potash. The juice from the stalks should therefore be returned to the land.

BLEACHING.

The operation of bleaching paper pulp as it is practiced under factory conditions, where time is the important factor, is difficult to approximate

in the laboratory. However, quantitative bleaching experiments allow of considerable accuracy and should afford fairly reliable data for comparative purposes under practical conditions.

Bleaching powder (chloride of lime) is used almost to the exclusion of all other agents, so that it alone need be considered. This chemical is usually applied in the form of a clear solution of approximately 5° Bé. strength (specific gravity, 1.036), made by disintegrating the powder in water and clearing it by allowing it to settle or by filtering. The amount of bleaching powder necessary to produce the required shade depends on several factors, chief among which is the completeness of the previous treatment—that is, the removal of the noncellulose.[12] The kind of fiber to be bleached also materially affects the amount of bleaching agent required. It is the practice to base all calculations as to quantity upon the amount of bleaching powder required to bleach 45.4 [13] kilos of the original fiber. The quantities in general use for this weight of different fibers are stated as follows:

	Kilos.[14]
Rags (cotton and linen fiber)	0.907– 2.268
Straw	3.175– 4.53
Esparto	4.53 – 6.80
Wood	5.44 –11.34
Jute and Manila	4.53 – 9.07

The pulp is bleached either in the beating machine or in large chests termed "plotchers." The exact procedure varies in the different mills, but time-saving devices and schemes are quite generally employed—that is, acid is added to hasten the bleaching action and artificial heat is supplied for the same reason. The following details of procedure, adopted for quantitative bleach experiments on abacá, apply as well to all the other fibers studied. It will be noted that all digestion experiments were performed on 1-kilo lots of raw material. This furnished in most instances about 450 grams of pulp. The washed, dry residues were repulped by beating or shaking with a definite volume of water, and a clear solution of bleaching powder of known strength was added, slightly in excess of the amount required to produce a good white. In some instances the operation was performed in a model beating engine of the Hollander type of about one-half kilo capacity, but most of the bleaching

[12]A well-boiled pulp should contain 90 to 95 per cent of cellulose—that is, will lose from 5 to 10 per cent in weight in the process of bleaching. Cross and Bevan: *Text-book on Paper Making*, 160.

[13] 100 pounds.

[14] 2– 5 pounds.
7–10 pounds.
10–15 pounds.
12–25 pounds.
10–20 pounds.

was done in 3-gallon glass jars provided with mechanical agitators. The experiments were conducted at ordinary temperatures (about 30° C.), and no attempt was made at "souring" by means of acids or of otherwise accelerating the action. The endeavor was rather to obtain a slow, regulated process which involved the least injury to the cellulose and a maximum yield of bleached pulp. After a good, white appearance was obtained to the beaten pulp, the stuff was filtered and the residual available chlorine determined by titrating an aliquot portion of the filtrate with a standard solution of thiosulphate. The filtering and washing of the bleached cellulose was performed by means of suction, a large Buchner funnel with small perforations, proving a very efficient mold in which to wash and press the pulp into discs for a final weighing.

BLEACHING ABACÁ PULP.

Experiment No. 1.—The dry residue from digestion No. 2, page 445, amounting to 328.4 grams, was placed in the engine and beaten to a homogeneous mass with 9 liters of water, then 2½ liters of clear bleaching powder solution, strength 2.35 per cent available chlorine (approximately 10° Bé.) were run in and the stirring continued until the pulp was of a light cream color. At this stage 100 cubic centimeters of the liquor required 31 cubic centimeters of N/10 thiosulphate, equivalent to 0.11 gram of chlorine, or to 12.1 grams of residual chlorine in the entire liquor. The amount of chlorine added (2½ liters of 2.35 per cent chlorine solution) was 58.75 grams. The difference between this quantity and the 12.1 grams remaining at the end of the bleach, amounting to 46.65 grams, represents the amount used in bleaching 324.8 grams of pulp or 1,000 grams of original waste. This is 14.2 per cent of chlorine, calculated on the weight of pulp, or 4.66 per cent calculated on the weight of the raw material originally taken. Using bleaching powder of 35 per cent available chlorine as a basis for calculating into terms of general usage, the above results are equivalent to 14.2 per cent of powder.[15] The dry, bleached residue weighed 304.4 grams, so that there was a loss of 7.3 per cent in the bleaching operation.

Experiment No. 2.—The residue from digestion No. 3, page 445, weighing 317 grams was employed. A bleach liquor of much less strength (1.65 per cent available chlorine, approximately 3½° Bé.) was used, otherwise the conditions were the same as in the preceding experiment. The amount of bleaching powder required was 12.22[16] per cent of the raw material. The dry bleached residue weighed 297 grams, so that there was a loss of 6.4 per cent in bleaching.

CHEMICAL INVESTIGATION OF FIBROUS SUBSTANCES IN GENERAL.

The schemes which have been used for the analysis of fibrous substances and the methods adopted for valuing unknown raw materials for paper stock are substantially those of Messrs. Cross, Bevan, and King, whose valuable works on fibers,[17] cellulose,[18] and paper making[19] stand

[15] 13.33 pounds per hundredweight of waste.

[16] 12.2 pounds per hundredweight of waste.

[17] *Indian Fibers and Fibrous Substances* (1887), London, Spon, E. and F. N.

[18] *Cellulose* (1895), London, Longmans, Green & Co.

[19] *Paper Making* (1900), London, E. & F. N. Spon.

preëminent in this interesting field of chemical research. The principal scheme of analysis used by these chemists is briefly, as follows:

	Moisture	Hygroscopic water, or water of condition. Loss on drying at 100° C.
Separate portion taken for each determination. (Results calculated in percentage of dry substance.)	Ash	Total residue left on ignition.
	Hydrolysis (*a*)	Loss of weight on boiling raw fiber five minutes in 1 per cent solution of caustic soda.
	Hydrolysis (*b*)	Loss of weight on continuing to boil one hour.
	Cellulose	White or bleached residue from following treatment: (1) Boiling in 1 per cent solution of caustic soda five minutes; (2) exposing to chlorine gas one hour; (3) boiling in basic sodium sulphite, 2 per cent solution.
	Mercerizing	Loss on treating one hour with 33 per cent solution of caustic soda, cold.
	Nitration	Weight of nitrated product obtained by treatment with a mixture of equal volumes of nitric and sulphuric acids one hour in the cold.
	Acid purification	Raw fiber boiled one minute with acetic acid (20 per cent) washed with alcohol and dried.
	Carbon percentage	Determined by combustion.

This method of study is of value in that it gives results which are dependent upon definite properties of the fiber substance, but it is more extended than is required for special or more practical purposes, therefore it has been restricted to our wants and also modified in some respects.

Moisture.—As is well known, all celluloses under normal conditions contain a certain amount of moisture which is termed "water of condition," which is quite definite for each fiber, although it varies within limits which are dependent upon the hygrometric state of the atmosphere. It is characteristic of abacá waste that it readily absorbs moisture and in an ordinarily dry condition holds from 11 to 13 per cent, but during the rainy months the water content was found to reach a maximum of 18 per cent. This hygroscopic moisture is a factor of some importance in the buying and selling of fibrous substances either in the raw state or as paper pulp. It is customary to decide on some arbitrary percentage as representing the moisture normally present. The standard used varies with different countries and with different kinds of material. With wood-pulp, in which there is considerable commerce, 10 per cent is usually taken as representing the moisture normally present.

Graffin and Little[20] give the following method for calculating the percentage of air-dry material: A representative sample of the substance to be tested is dried in a water-jacketed air bath at 100° to constant weight. The loss in weight represents the moisture in the sample and is calculated into percentage, the difference between this figure and 100 giving the percentage of absolutely dry fiber. On a 10 per cent basis every 90 parts by weight of dry material is equivalent to 100 parts of air-dry substance; on this basis, dividing the percentage of dried material formed by 90 and multiplying the quotient by 100 gives the percentage of air-dry substance.

Example: A sample of waste lost 15 per cent on drying, then 85 per cent represents the dried fiber; if the standard is 10 per cent, then $85/90 \times 100 = 94.4$ per cent,[21] or the percentage of air-dry fiber actually in the amount of material paid for on a 10 per cent basis.

The effect of this water of condition on commercial dealings in fibrous products is emphasized when values are considered. In the delivery of, say, 2,272.7 kilos of material at 4.4 cents per kilo,[22] the difference is 127.2 kilos or $5.60.

Ash.—"The ash in isolated fibers is low, viz, 1–2 per cent; in fiber aggregates it is often high—thus, in esparto and straw from 3 to 6 per cent—and should be taken into account in calculation of yields or loss of weight."[23]

I have found several samples of rice straw to average 18 per cent of mineral matter; but this is an extreme case. Griffin and Little give the range from 3 to 7 per cent, or in exceptional cases as high as 12 per cent on the dry straw, and further add that the strongest straw yields the most ash.

Alkaline hydrolysis.—"This is obviously the first stage toward the isolation of cellulose. When the numbers obtained for the short period (*a*) and for the long digestion (*b*) show a marked difference, it is an obvious general indication of low paper-making quality."[24]

Cellulose.—Cross and Bevan's chlorination method gives the maximum theoretical yield. Various other available methods give figures which are 2 to 5 per cent lower, but in comparison they consume much time. All the cellulose determinations recorded in this paper have been made on the residue from hydrolysis (*b*) which yields results more nearly in accord with other methods, at the same time avoiding their tediousness.

After this preliminary discussion of methods and their meaning, the results of the chemical investigation of abacá fiber will be given.

[20] *Chemistry of Paper Making*, 451.

[21] 94.4 pounds of normal, air-dry fiber per hundredweight.

[22] 2½ tons at 2 cents per pound.

[23] Cross and Bevan: *Text book of Paper Making*, p. 92.

[24] *Loc. cit.*

CHEMICAL INVESTIGATION OF ABACÁ FIBER.

Ash, alkaline hydrolysis, and cellulose calculated on dry weight.

	Per cent.
Moisture	8.10
Ash	1.08
Hydrolysis (*a*)	13.86
Hydrolysis (*b*)	20.79
Cellulose	73.68

Composition of raw abacá fiber (undoubtedly hand stripped).

[Analysis by Hugo Müller.[25]]

	Per cent.
Cellulose	64.07
Fat and wax	.62
Aqueous extract	.96
Lignin and pectous substances	21.60
Water	11.73
Ash	1.02

Figures obtained by myself on a sample of machine-stripped fiber.

	Per cent.
Cellulose	63.15
Fat and wax	.66
Aqueous extract	1.00
Lignin and pectous substances	24.67
Water	9.50
Ash	1.02

MICROSCOPIC CHARACTERISTICS OF ABACÁ FIBER.

Manufacturers of paper attach considerable importance to the physical condition of cellulose after it has been freed from foreign matter and otherwise purified by the boiling, washing, and bleaching processes to which it has been subjected.

Some fibers are short, hard, and of polished exterior, while others are long, flexible, and barbed; the former, it is scarcely necessary to say, yield but indifferent papers, easily broken and torn, while papers produced by the latter class of fibers are possessed of a great degree of strength and flexibility.[26]

According to Watt:[27]

Straw fiber is short, pointed and polished, and can not of itself make a strong paper; on the other hand, Manila (abacá) is the strongest known, and esparto fiber holds an intermediate place between these two extremes.

The dimensions of abacá fiber are included in the table given on page 461, together with those of the others examined. For purposes of

[25] Griffin and Little: *Chemistry of Paper Making*, p. 127.

[26] Arnot: *Journ. Soc. Arts*, **26**, 74.

[27] *Art of Paper Making* (1901), p. 4, Crosby, Lockwood & Son, London.

comparison the measurements of many well-known commercial fibers are also given.

The microphotographs depict the fibers both in transverse and longitudinal sections. The relative widths of the fiber walls and central canals as well as their rounded or polygonal forms and the shape of the tips, are clearly illustrated.

DIRT AND FOREIGN MATTER IN ABACÁ WASTE.

The cleanness of the raw material is an important consideration with paper makers. Foreign substances, such as sand, do not disappear in the pulping process and appear to be multiplied in the bleached and beaten half stuff.

In this respect abacá waste as it is ordinarily collected becomes very much contaminated. The greater part of this accumulation of foreign matter can be avoided if extra care is taken in the stripping of the fibers, but even then the waste would need to be thoroughly dusted at the factory and either picked over by hand or cleaned by machinery, according to the methods used for the preparation of dirty rags and rope.

The Philippine Islands are the home of real Manila paper stock. The qualities which give to abacá its prominent position among the fibers of the world—i. e., its strength and durability—make it a source of paper stock par excellence. The same conditions which confront paper manufacturers in other parts of the world are to be met here. The material must be collected from widely scattered localities, but with improved means of transportation it seems possible that this waste from abacá could be baled and shipped to market along with the cordage fiber. This will soon be the case when it becomes generally known that a product which has hitherto been a total loss will command from 1 to 2 cents a pound and the result will be to augment the revenues of the Manila hemp industry by millions of dollars annually.

PLANTAINS AND BANANAS.

Two other species of the family of plants to which abacá belongs, namely, the banana (*Musa sapientum*) and plantain (*Musa paradisiaca*), are worth considering in regard to their availability for paper stock. The Philippines and the Indian Archipelago are regarded as the regions richest in bananas.

> This highly important fruit grows in every province and inhabited island of the Philippines, both in the wild and cultivated state, and numbers over fifty species. The statistics regarding its production and area are interesting only as indicating the extent to which the natural, spontaneous growth has been supplemented and added to by such industry as the natives have exerted in setting out plants in the vicinity of their houses, which after planting receive very little, if any, attention in the way of real cultivation.[28]

[28] *Report of the Philippine Census* (1905), 4.

The entire cultivated area devoted to bananas in the Archipelago was reported as being 33,913 hectares (83,802 acres), upon which 11,078,600 bunches of the fruit were said to have been produced. This also represents the number of stalks which practically go to waste at the present time, for, after the fruit is harvested, they are simply allowed to rot on the ground. These data do not include the wild, nonedible species of plantains which are so common in all parts of the Islands.

Dr. Forbes Royle[29] in discussing plaintains says:

> Of the value of plantain fiber for paper making, there can, I conceive, be no doubt. Some paper, though unbleached, but excellent as far as substance and tenacity are concerned, was sent from India by Dr. Hunter in 1851. In 1846 Mr. May showed the author some beautiful specimens of note and letter paper made from plantain fiber. Mr. Routledge subsequently made some excellent paper, both of a tough and of a fine quality, from fibers of this species of Musa.

The Bulletin of the Royal Kew Gardens for August, 1894, contains a valuable summary of information relating to bananas and plantains from which the following brief extracts have been taken:

> In Jamaica a series of experiments, undertaken by Mr. Morris in 1884, showed that the plantain fiber (*Musa sapientum* var. *paradisiaca*) was whiter and finer than ordinary banana fiber and that it approached more nearly the fine, glossy character of Manila hemp. A banana stem weighing 108 pounds yielded 25 ounces of cleaned fiber, or at the rate of 1.44 per cent of the gross weight. A plantain stem weighing 25 pounds yielded 7¼ ounces of cleaned fiber, or 1.81 per cent of the gross weight.
>
> The experiments of ten years on a cultivation of from 400 to 480 acres in plantains has shown that, first, 700 to 800 stems are cut per acre per annum, either for fruit or in consequence of having been blown down by high winds or from disease or other reasons; second, if planted 8 feet apart and cut every eight months for stems alone an acre would give 1,400 to 1,500 stems each cutting, or about 4,500 in two years; third, plantain stems average 2½ pounds of clean and 1½ pounds discolored and broken fiber, the latter only fit for coarse paper. These results, however, were obtained with very imperfect machinery; fourth, the average weight of a plantain stem is 80 pounds.

Complete stalks of wild plantain from Tarlac Province, Philippine Islands, were found to average over 100 pounds in weight. One entire stalk was sawed into short cross sections and subjected to strong pressure. The dried residue was 9.3 per cent of the gross weight of the stem. Crude fiber estimation (Weende Method) gave 2.46 per cent of the gross weight of the stem. A second stalk was divided longitudinally into narrow strips. These were drawn under a knife edge, which removed the greater proportion of the water and cellular matter. The remaining thin, fibrous ribbons were boiled in a dilute solution of sodium carbonate containing a proportionate quantity of quicklime until the

[29] Royle: *The Fibrous Plants of India*, p. 87.

incrusting substances were dissolved. Thorough washing produced 1 kilo of light-gray textile fiber.

The following data are reported by Mariano Vivencio, of this Bureau:

Comparison of a stalk from an edible variety of banana with one from abacá.

	CRUDE FIBER.	WATER.
	Per cent.	Per cent.
Banana stalk (green)	2.21	91.48
Abacá stalk (green)	2.90	92.70

PULPING AND BLEACHING EXPERIMENTS ON STRIPPED AND UNSTRIPPED PLANTAIN FIBER.

Experiment No. 1.—Six hundred and eighty grams of the cleaned fiber, obtained by boiling the outer fibrous strips of plantain stalks in dilute sodium carbonate and lime (see p. 453) were digested under 5–6 atmospheres pressure for five hours, with 20 per cent caustic soda, calculated on the weight of the material. The washed, dry residue weighed 442 grams, equal to a yield of 65 per cent. This residue was repulped in an excess of water and bleached under exactly the same conditions as those used for bleaching abacá pulp. Chlorine consumed, 15.2 grams, equivalent to 43.4 grams of bleaching powder of 35 per cent strength.[30] The dry, bleached pulp weighed 404 grams, a loss of 8.6 per cent in bleaching.

The plantain fiber when it is extracted is of a white, silvery luster and contains many strong filaments. However, in this respect it is very inferior to abacá. This inferiority for cordage purposes proves to be an advantage when it is considered for paper stock, for the ease of resolution into the ultimate fibers is directly proportional to the tensile strength of the filaments.

CHEMICAL INVESTIGATION OF PLANTAIN FIBER.

Ash, alkaline hydrolysis, and cellulose calculated on dry weight.

	Per cent.
Moisture	10.02
Ash	3.46
Hydrolysis (*a*)	16.78
Hydrolysis (*b*)	27.42
Cellulose	68.21

Composition of raw fiber from plantain.

	Per cent.
Cellulose	61.68
Fat and wax	1.06
Aqueous extract	1.77
Lignin and pectous substances	22.79
Water	9.57
Ash	3.46

[30] 9.81 pounds of powder per hundredweight of pulp, 6.38 pounds of powder per hundredweight of raw fiber.

MAGUEY.

Agave cantula.

After abacá, maguey or sisal hemp may well be ranked as the second most important fiber-producing plant of the Philippine Islands. The maguey plant is widely distributed throughout the Archipelago and has long been used for local purposes.[31]

The structural fiber of this and of other species of *Agave* is used for cordage and like abacá, it finds its way to the paper mills in the form of old rope, binder twine, etc. The following figures were obtained on a specimen of machine-stripped maguey fiber:

Ash, alkaline hydrolysis, and cellulose calculated on dry weight.

	Per cent.
Moisture	9.23
Ash	1.96
Hydrolysis (*a*)	13.78
Hydrolysis (*b*)	15.70
Cellulose	77.05

The corresponding data on *Agave keratta* from the West Indies, given by Cross, are:

	Per cent.
Moisture	15.5
Ash	1.4
Hydrolysis (*a*)	10
Hydrolysis (*b*)	20
Cellulose	75.8

Composition of the raw fiber.

	Per cent.
Cellulose	70.09
Fat and wax	.71
Aqueous extract	1.54
Lignin and pectous substances	16.84
Water	9.03
Ash	1.79

MAGUEY WASTE.

The waste fiber employed for the following digestion experiments was taken during some machine-stripping tests which were made on maguey leaves from Vigan, Ilocos Sur, by the Bureau of Agriculture of the Philippine Islands. The possibility of materially reducing the weight and bulk of this waste material by boiling it with lime in the localities where it is produced suggested itself, and experiments to decide this question were first undertaken.

Experiment No. 1.—One kilo of partially cleaned, dry waste was boiled in an open kettle for twelve hours with 20 per cent of quicklime calculated on the weight of the material. The washed, dry residue weighed 685 grams, or 68.5

[31] *Report of the Bureau of Agriculture* (1904), 82.

per cent. The residue was unpulped, of a dark-brown color, and very coarse. Digested for five hours longer, under 6–7 atmospheres pressure with 10 per cent caustic soda calculated on the original weight taken. The yield was 31.5 per cent of a light-gray, well digested pulp.

Experiment No. 2.—Uncleaned waste; 20 per cent quicklime; time of digestion, five hours; pressure, 6–7 atmospheres; yield, 55 per cent of a coarse, brown residue only suitable for cheap wrapping paper.

Experiment No. 3.—Time of digestion, twelve hours; all other conditions as in experiment 2; yield, 38 per cent. The residue appeared to be well pulped, but not entirely free from cellular matter.

Experiment No. 4.—Twenty-five per cent caustic soda, calculated on the weight of material digested; time, five hours; pressure, 7 atmospheres; yield, 35 per cent; residue, light-gray, and it appeared to be very well boiled.

Experiment No. 5.—Twenty per cent caustic soda; time, five hours; pressure, 7 atmospheres; yield, 41.5 per cent; residue, dark-gray; digestion, incomplete.

Experiment No. 6.—Twenty per cent caustic soda; boiled twenty-four hours at ordinary pressure with reflux; yield, 50 per cent; residue similar to that from experiment 2.

Conclusions.—For the preparation of half stuff for brown wrapping paper the waste may be lime boiled under pressure with good results both in respect to yield and to economy; however, an open cook with lime gave negative results. The weight of the waste may be reduced by about 30 per cent by this latter procedure, but the resulting product is not in any sense a paper pulp. Caustic soda (25 per cent calculated on the gross weight of the material) effects a complete re-solution of the incrusting material.

BOWSTRING HEMP.

Sanseveria zeylanica is found quite widely distributed throughout the Islands and is cultivated in some localities for cordage and textile purposes. It is mentioned in this place because of the similarity which its structural fiber has to that of maguey and because of the possibility of its becoming a plant of considerable commercial importance. Like maguey, it readily lends itself to machine stripping and in the event of a more extensive cultivation the waste fiber may be worth considering.

GRASSES IN GENERAL.

Gramineæ.—Among the *Gramineæ* are many well-known annual and perennial plants which have in the past, largely been used in the manufacture of paper; the cereal straws, such as those of wheat, rye, oats, and barley, and the stalks of maize and of sorghum, are examples of materials from annual plants which are used for this purpose. Esparto and the British Indian grasses *Munj* and *Bhabur* may be mentioned among the perennial plants, as they have assumed a considerable commercial importance. The various species of bamboo have not as yet, because of the reasons stated on page 434, been industrially established.

Esparto.—The Esparto grass (*Stipa tenacissima*) is found both cultivated and in the wild state in the countries bordering on the Mediter-

ranean, principally in Spain and in northern Africa. In the regions where this grass grows the material is used for the manufacture of cordage, sandals, tie material, and basket work, but its great commercial importance lies in its use for paper stock. The cost of its production and of its shipment have excluded the use of esparto in the United States, where wood pulp has been so cheap and plentiful, but in Great Britain it has found a steady market, approximately 200,000 tons, valued at from $15 to $20 per ton, being imported annually into that country.

Bhabur grass.—The Bhabur grass (*Ischaemum augustifolium*) which is met with throughout the central table lands of India from Bengal to Madras, the northwest provinces and central India, is in some parts extremely abundant. As a paper material this grass has been reported as little inferior to esparto and the paper made from it is of good quality. The Indian paper mills use Bhabur grass largely.[31]

The Munj grass (*Saccharum sara*), which is common from Bengal to north India, as well as several other species of *Saccharum*, are largely used in the upper India mills near Lucknow. Indeed, this is one of the most valuable of Indian fiber materials, in some respects being superior to Bhabur grass. The great obstacle to its further use is the difficulty of procuring a large and constant supply.[32]

The following statistics which refer to the paper industry of India are especially significant in their bearing on the possibilities of paper production in the Philippines, as the raw materials in general use in the mills of India are very similar to those which we are about to discuss:

There are eight paper mills in operation, two of which are private concerns. The capital invested so far as information is obtainable is rupees 7,320,000 (approximately $2,500,000). Most of the white and blue foolscap and much of the blotting paper, note paper, and envelopes used in the Government offices are now made in the Indian mills.

The total quantity of paper made in 1902 was 47,000,000 pounds, valued at $2,092,000, approximately.

The capital has trebled in twenty years since 1883 and the production and number of persons employed has increased sixfold during the same period.[33]

PHILIPPINE GRASSES.

Cogon.—Two grasses, Cogon and Taláhib, are found in the Philippine Islands growing wild and apparently in sufficient quantities to warrant some inquiry as to their economic use. In the Philippines many coarse grasses are termed cogon, but, properly speaking, Cogon (*Imperata exaltata* Brong.) is the broad-leaved, gregarious variety, 2 to 4 feet in height, which grows in even stands on open lands, foothills, and mountains. Where nipa is not to be obtained the true cogon is highly prized for thatching purposes. Conditions of drought do not appear to affect

[31] Watt: *Dictionary of Economic Products of India*, **6**, part 1.
[32] *Ibid.*
[33] *Indian Forester* (1904), **29**, 482.

its growth, as it seems to thrive on a soil containing but little moisture; indeed, even in the driest season it will spring up in a green and thrifty manner after being burned over, while, on the other hand, it is not found along river bottoms or on lands subjected to overflow during the rainy season. The study of cogon grass was conducted in conjunction with that of esparto in this laboratory because the use to which it is proposed to put the former is identical with that established for the latter. A direct comparison is therefore important and necessary. We were furnished with the Spanish variety of esparto from Barcelona through the kindness of Don Enrique Zobel, of Manila, and of the Compañía General de Tabacos de Filipinas. Our thanks are due for the great courtesy.

Ash, alkaline hydrolysis, and cellulose calculated on dry material.

	COGON GRASS.	ESPARTO GRASS (SPANISH).
	Per cent.	Per cent.
Moisture	9.32	8.68
Ash	4.53	3.71
Hydrolysis (*a*)	25.77	19.75
Hydrolysis (*b*)	40.00	37.16
Cellulose	50.11	55.16

Chemical composition of cogon and esparto grass.

	Per cent.	Per cent.
Cellulose	46.68	48.25
Fat and wax	1.16	2.07
Aqueous extract	10.80	10.19
Pectous substances	26	26.39
Water	11.33	9.38
Ash	4.03	3.72

Experimental work with mature cogon grass, the results of which are given below, indicates, first, the yield of paper cellulose to be expected under factory conditions of treatment; second, the approximate amount of caustic soda necessary to produce a well-boiled pulp; third, the comparative, resistant nature of the cellulose to further hydrolysis.[35]

Percentage of—	Experiment 1.	Experiment 2.	Experiment 3.	Experiment 4.
Soda liquor	2	2.4	2.5	3
Caustic calculated on weight of material	10	12	12.5	15
Yield of pulp	47.34	45.5	45.42	44.25

[35] Well-dusted grass, free from weeds and root ends, was cut into pieces 2 to 3 inches long and in this condition placed into the autoclave. Dilute caustic-soda solution in amount sufficient to cover the grass was then run in and the pressure was rapidly raised to 4 atmospheres as a maximum. One kilo of dry grass was taken for each digestion; the duration of the boiling was six hours.

All four of the pulps were light-gray in color and they appeared to be well reduced. The digestions were carried out in an autoclave built of bronze and the heating was done with direct fire. By the use of patent boilers of the vomiting type and by heating with direct steam (improvements which are in general use with esparto grass) results equal to those given above could no doubt be brought about in a much shorter time. The pulp has a great tendency to form little balls which are very similar to those encountered with esparto and straw, hence rotary boilers would not be suitable. The modern tendency is either to increase the pressure or the amount of caustic soda used, thus diminishing the duration of the digestion.

Two digestions with 2 per cent liquor, but with 20 per cent caustic soda, calculated on the weight of the material, were carried out at 4 to 5 atmospheres pressure for three hours. The yields of pulp were 44 and 45 per cent, respectively.

In some cases, esparto and straw are boiled in open kettles, but if this is done, a much longer time is required to accomplish the same result. The following table gives the results of two such experiments:

Digestion of cogon at ordinary pressures.

	I.	II.
Strength of liquor (per cent)	2.6	3
Caustic soda (per cent)	13	15
Time of boiling (hours)	12	12
Yield of pulp (per cent)	51.25	48.81

These results show that cogon could be boiled in open digesters with good results, to make the cheaper grades of printing paper, where time is not as important a factor as the cost of the process. The use of quicklime, the chemical so commonly employed in the manufacture of strawboard, did not give satisfactory results with cogon grass because the lime seems to combine with the soluble constituents from the grass and it then becomes extremely difficult to wash out. The pulps produced in this manner in the laboratory were coarse and brown. However, for the cheapest printing paper and for boards, a lime boiling in an open cook, or perhaps under 1 to 3 atmospheres pressure, would effect great saving in the cost of the chemicals to be used.

The investigation of cogon also proved that no preliminary cutting down of the grass is necessary; after being dried and hand picked or machine cleaned it is fit for immediate digestion.

THE BLEACHING OF COGON GRASS PULP.

Experiment No. 1.—The residue from digestion No. 3, page 458, amounting to 454.2 grams, was repulped in 10 liters of water; 1 liter of a bleaching solution of 2.35 per cent available chlorine (approximately 10° Bé.) was added and the

whole allowed to bleach over night. No residual chlorine remained, but the pulp was of a clear, white color. The amount of chlorine consumed was 23.5 grams (1,000 cubic centimeters of 2.35 per cent solution). This represents 67.1 grams of powder, equivalent to 6.71 per cent.[36] The bleached pulp weighed 428.7 grams, which gave a loss of 5.62 per cent in bleaching.

Experiment No. 2.—A residue from digestion with 20 per cent caustic soda, page 459, amounting to 440 grams, was submitted to exactly the same conditions as those given above. The residual chlorine amounted to 0.78 gram, which leaves 22.72 grams as the amount consumed. This represents 64.9 grams of powder.[37] The bleached pulp weighed 418 grams which represents a loss of 5 per cent in bleaching.

TALÁHIB, Tag., *Lidda*, Ilo., *Tigbao*, Vis. (*Saccharum spontaneum*).

This is a coarse-jointed grass, 6 to 10 feet in height, gregarious, growing in tufts from stout underground rootstalks. It is found to be distributed from British India to southern China, Malasia, New Guinea, the Philippine and Caroline Islands. It is very common throughout the Archipelago and is frequently confounded with cogon grass, but it is very distinct from the latter in appearance and in its habit of growth. It thrives best in valleys and in low, moist places, especially in areas which are flooded during the rainy season. It is very persistent and difficult to eradicate; simple cutting or burning does not appear to decrease its growth but rather to improve its yield and quality. Other related species are *Saccharum sara* (the Munj grass of India) and *Saccharum officinarum* (the well-known sugar cane of all tropical countries). A sample of mature grass consisting of entire stems and leaves gave the following figures:

	Per cent.
Moisture	10.23
Ash	5.46
Hydrolysis (*a*)	27.44
Hydrolysis (*b*)	40.53
Cellulose	53.90

If this grass is allowed to become too dry before cutting, the nodes harden and prove difficult to pulp, and a considerable quantity of shive is the result, so that some mechanical crushing or breaking of the stems is necessary. Only one digestion experiment was made. The grass was cut into short pieces and boiled in water for three hours to soften the nodes, then digested during six hours with 12½ per cent of caustic soda, calculated on the weight of the grass. Pressure, 4 to 5 atmospheres. Yield of pulp was 45.57 per cent. The residue bleached to a good white with only 3.2 per cent loss in weight by the use of 5.7 per cent of bleaching powder, calculated on the original weight of the material digested.

[36] 6.71 pounds of bleaching powder per hundredweight of grass.
[37] 6.43 pounds of bleaching powder per hundredweight of grass.

DIMENSIONS OF THE ULTIMATE FIBERS OF SOME PHILIPPINE FIBER PLANTS.

[Table by Dr. E. B. Copeland. All measurements are in millimeters.]

Name.	Length.			Diameter.					
				Total.			Lumen.		
	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.
Abacá (*Musa textilis*)	6.00	3.98	2.45	0.021	0.017		0.009	0.0066	
Plantain (*Musa sapientum* var. *paradisiaca*)	7.30	5.19	4.15	.026	.020	0.018	.016	.0106	0.007
Maguey (*Agave cantula*)	4.93	2.38	1.00	.026	.018	.015	.013	.007	.004
Cogon grass (*Imperata exaltáta*)	1.82	0.99	0.46	.021	.011	.005	.013	.0044	.001
Taláhib grass (*Saccharum spontaneum*)	2.82	1.59	0.80	.020	.015	.012	.010	.0043	.1115
Bamboo (*Bambusa blumeana*)		1.81			.0075				
Dwarf bamboo (*Bambusa lumampao*)	4.10	2.57	1.20	.028	.0156	.005	.025	.018	.001
Rice straw (*Oryza sativa*)		0.88			.0025				
Buri palm (*Corypha umbraculifera*)	2.80	2.11	1.10	.015	.013	.010	.007	.0031	.002

Table of dimensions of some of the more important fibers used in the manufacture of textiles and paper.

[Taken from M. Vetillart.[38]]

Name.	Length of fiber cell.			Diameter of fiber cell.		
	Maximum.	Mean.	Minimum.	Maximum.	Mean.	Minimum.
Flax (*Linum usitatissimum*)	66.0	25–30.0	4.0	0.037	0.022	0.015
Hemp (*Cannabis sativa*)	55.0	22.0	5.0	.050	.022	.016
Jute (*Corchorus olitorius*)	5.0	2.0	1.3	.025	.020	.020
Esparto grass (*Stipa tenacissima*)	3.5	0.5	1.3	.018	.0125	.007
Spanish grass (*Lygeum spartum*)	4.5	2.5	1.3	.020	.016	.012
Bowstring hemp (*Sansevieria* sp.)	6.0	3.0	1.5	.026	.020	.015
Paper mulberry (*Broussonetia papyrifera*)	25.0	15.0	6.0	.035	.030	.025
Tallpot palm (*Corypha umbraculifera*)	5.0	3.0	1.5	.028	.024	.016
Coconut palm (*Cocos nucifera*)	1.0	7.0	0.4	.024	.020	.012

We are greatly indebted to Mr. Elmer D. Merrill, Botanist of the Bureau of Science, for the collection and botanical identification of fibrous plants discussed in this paper and for other valuable assistance.

[38] *Etudes sur les Fibres Vegetales Textiles.*

EXPLANATORY NOTE ON THE SPECIMENS OF PAPER INSERTED IN THIS ARTICLE.

In response to numerous requests for specimens of paper made from the raw materials investigated, we have inserted into this number of the JOURNAL samples of handmade paper from abacá waste and cogon grass. Similar specimen sheets made from banana, bamboo, palms, and other material will be sent on request. It was thought best not to alter the pulp in any way, but only to provide pure bleached specimens, thus affording paper manufacturers and others who are interested an opportunity for testing the fiber to their own satisfaction. Consequently, no attempt at loading, sizing, coloring, or calendering the specimens was made. The work required to turn out several reams of handmade paper with apparatus available in the laboratory is very great, and it was practically all performed by Filipinos. This speaks favorably for their skill in acquiring facility in manipulations of this kind.

In a future paper other Philippine paper materials will be discussed, and data in regard to the available supply of all raw materials, so far as such data can be obtained, will be given.

ILLUSTRATIONS.

[Photomicrographs by Martin.]

PLATE I.

ABACÁ (*Musa textilis*).

FIG. 1. Transverse section of isolated fibers.
2. Fibers seen longitudinally.

PLATE II.

PLANTAIN (*Musa paradisiaca*).

FIG. 1. Transverse section of group of fibers.
2. Fibers seen longitudinally.

PLATE III.

MAGUEY (*Agave cantula*).

FIG. 1. Transverse section of a group of fibers.
2. Fibers seen longitudinally.

PLATE IV.

COGON (*Imperata exaltata*).

FIG. 1. Transverse section of a bundle of fibers.
2. Fibers seen longitudinally.

PLATE V.

TALAHIB (*Saccharum spontaneum*).

FIG. 1. Transverse section of isolated fibers.
2. Fibers seen longitudinally.

PLATE VI.

ESPARTO (*Stipa tenacissima*).

FIG. 1. Transverse section of isolated fibers.
2. Fibers seen longitudinally.

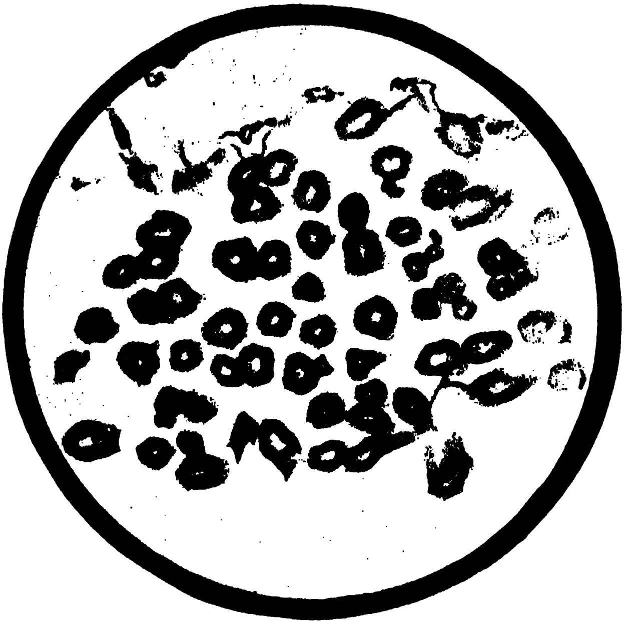


FIG. 1.

FIG. 2.

PLATE I.

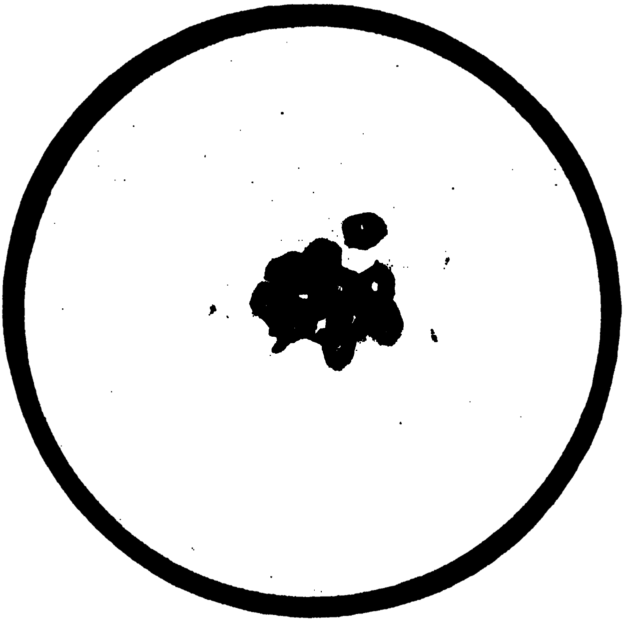


FIG. 1.

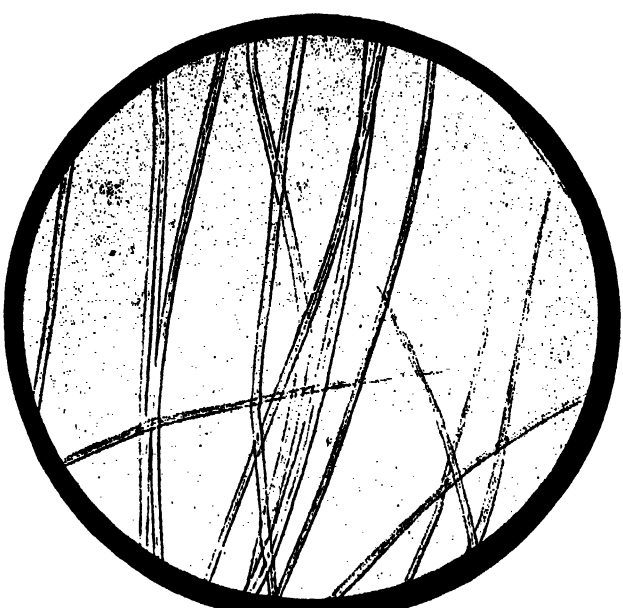


FIG. 2.

PLATE II.

FIG. 1.

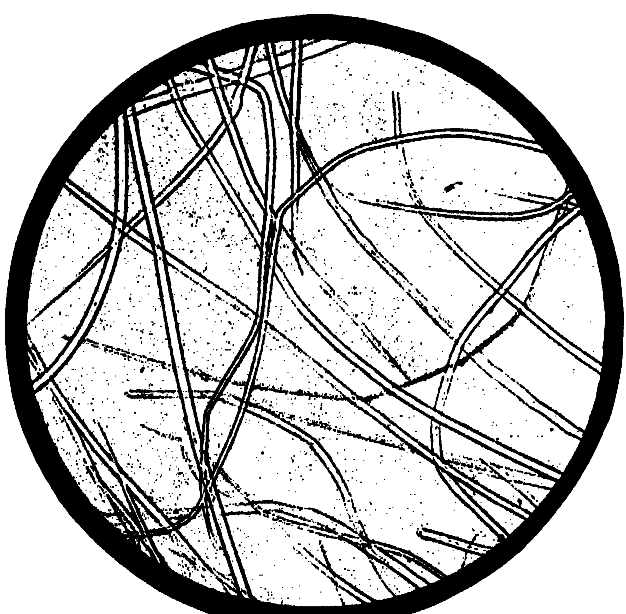


FIG. 2.

PLATE III.

FIG. 1.

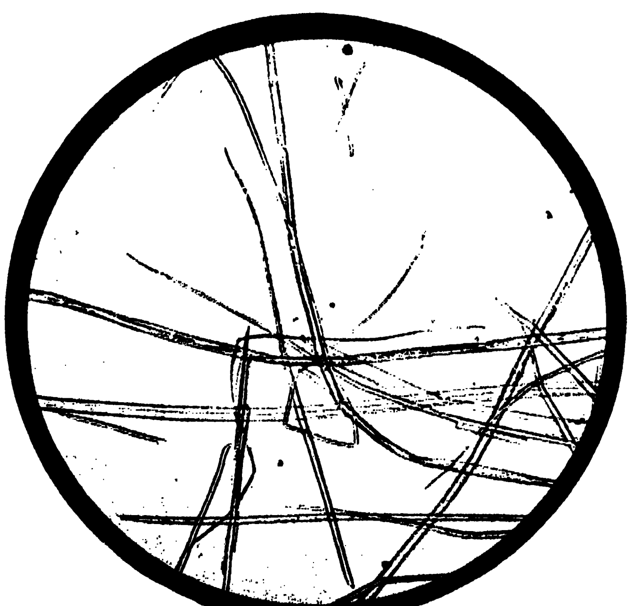


FIG. 2.

PLATE IV.

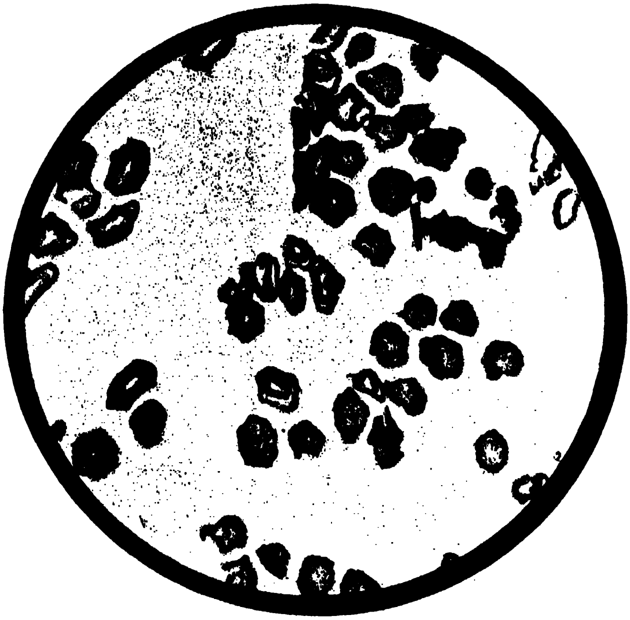


FIG. 1.

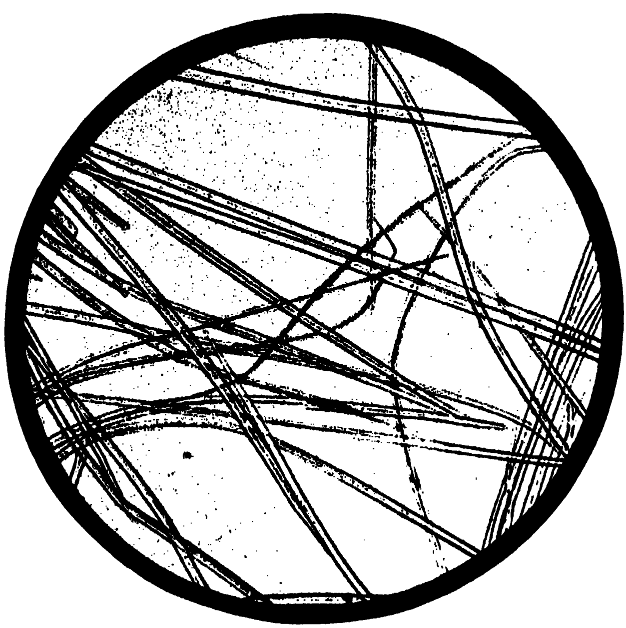


FIG. 2.

PLATE V.

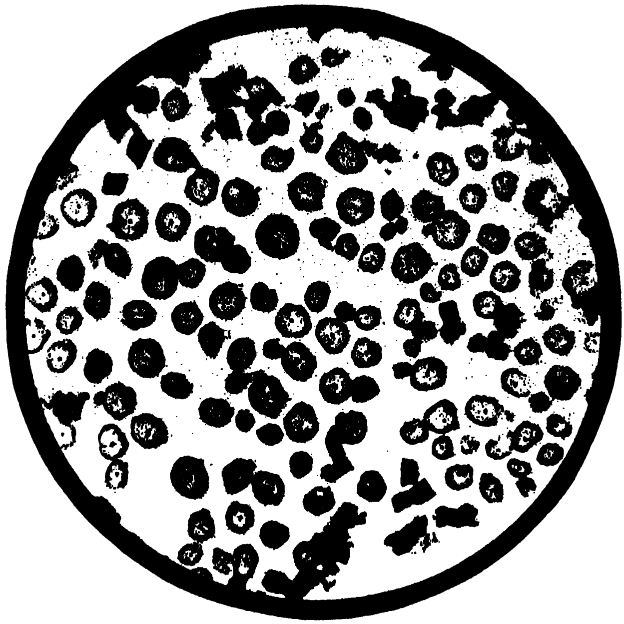


FIG. 1.

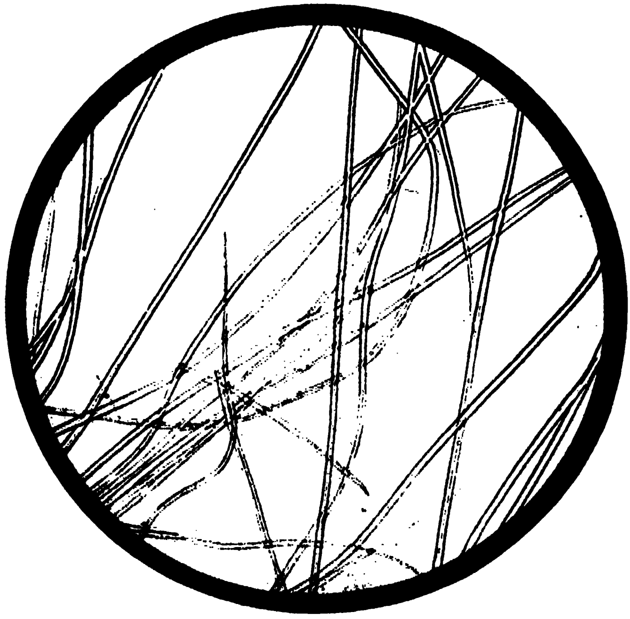


FIG. 2.

PLATE VI.

ABACÁ WASTE PAPER

COGON GRASS PAPER

COMBATING PLAGUE IN JAPAN.[1]

By SHIBASABURO KITASATO.

(From the Institute for Infectious Diseases, Tokyo, Japan.)

PLAGUE EPIDEMICS AND THEIR DETRIMENTAL EFFECTS.

In 1896 the epidemic of plague which had previously raged in India and Hongkong invaded Formosa, which from that time became a source of the dangerous pestilence. In this insular possession of Japan, a condition was produced in which the eradication of the etiological factor became almost impossible, and the situation began to menace the mother country. However, strict enforcement of preventive measures and quarantine precluded the entrance of plague from this into the main islands of Japan, but danger to our country was present from other sources, for the first case of plague encountered during this epidemic was found aboard a vessel which entered the port of Yokohama in 1896. The ship brought the dangerous germ from India and from southern China, with which regions Japan has frequent commercial intercourse. Since this event, steamers arriving at the same port and at Nagasaki and Kobe have brought in several cases of bubonic plague, but, as the discovery has always been made in time and preventive measures and quarantine have been promptly applied, infection has been avoided for the time being.

However, the origin of the epidemics in Japan has been located not in the patient but in inanimate objects. Incoming vessels from the infected regions—that is, from Bombay and Hongkong—began to introduce into the country the plague germ mingled with their freight, which principally consisted of cotton. The Government being unaware of this dangerous importation, the freight was allowed to land and as a consequence the infection rapidly spread in the principal trading ports. At first the epidemic prevailed among rats; afterwards it attacked human beings, with the result that many human lives were sacrificed, tens of thousands of yen of State money were expended, and the foreign trade of the country also suffered considerably.

The principal epidemics in Japan have been as follows: The first outbreak was that during the years 1899 and 1900. It began in Kobe;

[1] Read at the third annual meeting of the Philippine Islands Medical Association, March 3, 1906.

spread to Osaka, where its ravages reached a high mark, and then invaded Hamamatsu and Wakayama. The total cases in this epidemic numbered 230.

The second invasion was in 1902 and 1903, the source of this epidemic probably being in cotton which was imported from Hongkong. The initial cases occurred in Yokohama and it then spread to a district in Tokyo. The epidemic was stopped by the combined efforts of the two cities after it had claimed 71 victims.

The third outbreak occurred in 1905 and is still prevailing. Throughout different localities—namely, in Tokyo, Chiba, Kobe, Osaka, Kagawa, and Moji—a number of victims have succumbed to it. The disease appeared in Tokyo in the early spring, chiefly in the Fukagawa district, and it then spread to the Chiba perfecture, where a summer epidemic prevailed to a slight degree. The origin of the outbreak in Kagawa, lasting from May to the middle of June, appears to have been similar to that of the Osaka and Kobe epidemic and it was evidently due to direct contagion; its severity has been unparalleled in recent years. Two plague patients were found in the city of Kobe in August and the number of cases increased rapidly within the next few months, so that during the year the total number of victims amounted to 90. Next to Kobe, Osaka suffered most severely, there having been many cases since October of the past year, the number reaching 134 in three months. In both cities the outbreak spread throughout all the districts and it is still raging. Plague has also appeared in Shimonoseki and has claimed nine victims in that city. This epidemic has had no precedent both in its severity and in the number of its victims, the total of the cases during the year having reached 297.

The following table shows the regions of Japan in which the series of outbreaks occurred and the number of patients in each:

	Year.	Patients.	Deaths.	Principal regions of the epidemic.
First outbreak	1899	62	45	Kobe and Osaka.
	1900	168	158	Kobe, Osaka, Hamamatsu, Wakayama, and Nagasaki.
	1901	2	2	Wakayama.
Second outbreak	1902	14	9	Yokohama and Tokyo.
	1903	57	49	Do.
Third outbreak	1904	1	1	Kobe.
	1905	297	257	Tokyo, Kobe, Osaka, Chiba, Kagawa, Moji, and Nara.
Total		601	518	

The number of patients during the past year, distributed in different localities, is as follows:

Tokyo, 15; Chiba, 11; Osaka, 134; Nara, 2; Kagawa, 36; Kobe, 90; Moji, 9; total, 297.

In Japan the cost of preventive measures and quarantine has reached an enormous figure. During the first outbreak the city of Osaka expended more than 352,500 yen; during the second, Tokyo city spent 320,000 yen, although the number of patients there only numbered 15—i. e., 21,333 yen for every victim. These figures show the expense of plague outbreaks even when they are considered apart from their dreadful effect upon human lives. From the facts already given it will not be difficult to infer how much the present epidemic, which is raging in Kobe and Osaka with unparalleled vigor, will burden the financial resources of the country. Already the city of Kobe has given out 310,000 yen and Osaka city 470,000 yen for its prevention; and it is apparent from the present conditions that we will have to spend considerably more money to keep the plague at bay.

Such is the direct burden of plague upon the finances of a country, but this is not all; for the indirect detrimental effects must also be considered. This loss, indeed, can not be estimated, because it is a wide and far-reaching one, affecting both domestic and foreign affairs and one which can not be compared with that of an epidemic of any other kind which involves only its direct damage upon a limited community. Plague, indeed, is a fearful enemy to mankind.

Two methods of invasion are apparent from the studies of the epidemics in Japan: One is contagion from imported plague patients and the other by contact with the disease germ mingled with the freight brought in from some infected region. The nature of the preventive measures to be employed depends upon the source of the epidemic. If the invasion be by means of a plague patient, discovery is made easier and prevention or quarantine, as the case may be, can promptly be applied, so that the depredations of the disease may be confined within a small radius. On the other hand, if the organism is being propagated through the medium of rats, preventive measures are difficult: for, by the time infected rats are discovered, human beings have already become victims. Moreover, as a rule, by the time man receives the contagion from rats, the ravages of an epidemic have already reached serious proportions among these animals, so that the outbreak soon assumes a character which renders control difficult; the infection therefore spreads far and wide, affecting both men and animals. Such a result is illustrated by the first outbreak and by the present one in which Nagasaki and Kobe are the chief sufferers. The results at Chiba and Kagawa can be taken as an example of a case where the source of the epidemic was in human patients. In this outbreak we were able to keep the epidemic confined within a small locality by promptly applying the usual preventive measures—that is to say, before the rat was attacked by the germ. We can thus see that the same preventive measures may give different results in different cases.

In every epidemic it is difficult to ascertain the exact circumstances

under which the plague invasion occurs. This is especially true when the medium of propagation is outside the human patient, and it is notably the case where rats are the agents. So far, in every case, the plague outbreaks in Japan were first evidenced in localities which have direct communication with foreign countries and from these points the spread to the regions of the interior occurred. Moreover, the first case in any place which subsequently became a source of plague was invariably associated with freight imported from India or from Hongkong. The first epidemic owes its origin to a steamer which entered the port of Kobe with a cargo of raw cotton and Chinese rice from Bombay and Hongkong. The second one may be traced to a vessel which came to the port of Yokohama with freight consisting of raw cotton. The present great epidemic which prevails in Osaka and Kobe is due to a steamer which anchored in Kobe Harbor laden with a consignment of raw cotton from Bombay. These statements demonstrate the almost identical origin of the fatal pestilence in each instance.

As a rule, the plague germ on entering these trading ports does not immediately attack man; it first infects rats and by the time the first human victims are discovered, the epidemic has assumed a well-advanced form; this fact has been demonstrated on several occasions. The present epidemic in Osaka may be considered as an example. In February it was preceded by the discovery of infected rats, but only during May of the same year did human victims begin to appear, thus showing how deep rooted was the source of the infection.

The season during which an epidemic occurs has a marked influence. Thus it is apparent that a winter outbreak is generally of a most chronic character; it rages for a long period with great severity, spreading over a large extent of territory. The first invasion, the present epidemic in Osaka and Kobe, and the second one in Tokyo and Yokohama, belong to this class. A summer epidemic is usually acute in character and as a rule is spread by contagion. It may be severe though of short duration, and it is limited to a small area. The epidemics in Chiba and Kagawa during the past year and that of Hamamatsu in 1900 were of this nature. It should be noticed that during a winter epidemic a large number of infected rats are met with, while in a summer one but a small number or even no infected rodents are encountered. (See Pl. I.)

PREVENTIVE MEASURES.

As the plague epidemics in Japan, according to observation, originated in the Indian Peninsula, in Southern China, and in Formosa, the first and most urgent step to prevent the introduction of the disease was to establish quarantine against vessels coming from these regions. With this end in view, quarantine stations were erected at Yokohama, Kobe, Nagasaki, Moji and other principal trading ports and the necessary measures enforced as strictly as possible. So far, several cases of plague

have been discovered on board vessels coming from the infected regions; but as a result of the strict quarantine, the danger of contagion has been prevented. When a ship is found to have plague infection on board, all communication with the land is stopped, measures are taken to destroy the rats, and other sanitary precautions are employed. In order to provide the means for killing rats on board ship, auxiliary vessels especially fitted for that purpose and similar to those used in Hamburg, Germany, are recommended. We now have several of these at Yokohama, Kobe, and Moji, and they will be employed during the present year.

The Department of the Interior of the Japanese Government, through the Bureau of Public Health which belongs to that Department, names officials who are to meet to discuss and execute measures for the prevention of infectious diseases. These committees consist of physicians and surgeons with assistants and quarantine commissioners. Each municipality or prefecture has also its established board of health, which consists of one physician, several assistants, and a number of inspectors. This organization is placed directly under the control of the police department, and it takes charge of all matters relating to infectious diseases. The principal prefectures and districts are provided with isolating hospitals to receive patients and with laboratories for investigation and these boards of health carry out the necessary preventive measures. In case of a plague outbreak, the prefectural government increases the number of health officials so as to meet the emergency. When an epidemic of an unusual nature takes place, an imperial receipt is issued by means of which the organization of a special board consisting of commissioners, inspectors, clerks, and watchmen is ordered for fighting plague. In addition, several councilors are appointed to submit advice relating to the matter. The municipal government of Osaka has increased its staff by 311 officials and a number of councilors because of the present outbreak.

In Japan all affairs relating to health and sanitation, particularly to the prevention of infectious diseases, are referred to local administration, the execution of the necessary measures being carried out by the aid of the police. The expenses required for the purpose are shared by the people of the locality in which the disease occurs, aid also being given from the financial resources of the prefectural or municipal government. In case of an outbreak of contagious disease, the Central Government will give assistance to the local ones to an amount not exceeding one-sixth of the total outlay. However, in the case of plague, the Central Government has spent and is spending an amount enormously in excess of that fixed by regulation.

The Institute for Infectious Diseases acts as councilor to the Government in matters relating to health, and it especially advises in regard to the preventive and sanitary measures which are to be taken against

infectious diseases. When a plague epidemic exists, our Institute, through governmental appointment, furnishes those commissioners and officials who are to take charge of the preventive and sanitary measures. It has a plague laboratory constructed in conformity with the most advanced methods and in this place pest serum and vaccine are prepared for the use of the entire country. As a part of its work the institute gathers a number of physicians and surgeons throughout Japan in order to give them instruction in bacteriology and epidemiology. The number who have finished this course at present amounts to 1,293, and these physicians are scattered throughout the country. At least a portion of them are now actually engaged in important work in hygiene and preventive medicine. As these specially educated physicians and surgeons are distributed throughout Japan, it is a matter of ease in an emergency at once to gather several hundreds of commissioners. To fight plague successfully requires a large number of trained men, and the country owes its thanks to the Institute for rendering so many available.

Such, in brief, is the account of the organization of the preventive work which so far has been instituted in Japan. However, I must here express my sincere admiration and surprise at the manner in which your country is protected from pestilence. You have medical officials stationed at the principal ports of the world which plague frequently haunts, so that inspections are made at the places from which vessels clear. I believe preventive measures against plague to be the most urgent need of the age, but in order to be of permanent good they must include, as is the case with the United States, not only temporary means for preventing the intrusion of the disease to be enforced at the ports of entry, but also medical officials to be stationed in infected regions, at which points any vessels starting for the country in question can be strictly examined. I am of the opinion that it would be advisable to place such officials at Indian and South China ports to which the plague outbreaks in Japan have been traced.

In combating plague, the quarantine of ports, however strict and complete it may be, can not safely be relied upon and for this reason general provision throughout the country must be perfected against infectious diseases. As plague can not be classified with the ordinary ones of this class, the regulations to meet such emergencies in Japan were found in general to be unsuitable, so that the Government was compelled to remodel them in order to meet the condition. The principal features of the new regulations are as follows:

1. Authority for disinfection, isolation, and quarantine is given. Measures looking to the prevention of the disease are to be enforced not only against the living patients and in respect to the houses and furniture occupied and used by those actually stricken with plague, as well as of those who are only suspected of having the infection, but also in regard to the bodies of those dead with plague.

2. To prevent the spread of the infection, as many rats as possible are to be killed.

3. The period of isolation of suspected patients is not to be less than ten days.

These modified regulations were based upon the experience of several years. In carrying out measures of disinfection it is necessary to extend the area to be covered as far as possible, as the plague germ is very easily propagated. It is also true that bubonic plague often develops into that of the pulmonary or pneumonic type and in that event the disease not infrequently infects by direct contagion therefore, especially in times of epidemic, quarantine and isolation must be strictly enforced. It was thought necessary to fix the time of isolation at ten days because of the fact that the incubation period of the disease lasts from six to seven days. The killing of all rats which it is possible to secure was deemed a necessity, as these animals have the closest connection with plague. The above principles were applied where the intrusion of the pestilence was suspected even before the formulation of the new regulations.

In attempting to catch rats it is important to remember that traps and appliances should be set in all buildings, and it is imperative in those which contain cotton and grain, in which the plague germ is most liable to be retained. I gave warning in this respect a few years ago and as a result the municipal government of Tokyo has passed an ordinance respecting buildings and warehouses which provides that all buildings must be constructed in a manner to prevent their becoming an abiding place for rats. A similar regulation has been adopted in other cities and prefectures, especially in those having direct connection with foreign countries. These legal acts appear to be rather despotic, but nevertheless they must be deemed necessary in view of the dreadful character of the disease which is propagated by these rodents. I believe that the extension of similar regulations to dwellings so as to render all of them rat proof would in the future generally lessen the danger from the infection. Such a step has yet to be taken.

SANITARY WORK.

The principal undertakings established for the enforcement of preventive measures are (1) bacteriological examinations; (2) search for patients; (3) killing of rats, disinfection, and the application of sanitary methods; and (4) disposal of patients and of infected articles. The work may briefly be described as follows:

The bacteriological laboratories in the different districts and prefectures are for the study and diagnosis of plague, and in times of epidemic they are used solely for this especial purpose. In such cities as Kobe, Osaka, Yokohama, and Tokyo, which are most frequently threatened by pest, the Government for several years past has been encouraging

the destruction of rats by buying all which are killed or captured by the people. This practice has a twofold purpose—one the prevention of an epidemic, and the other the ascertaining whether any infected animals, the presence of which usually precedes an epidemic among men, are existent. An immense number of rats is bought by the Government and each one of them is examined bacteriologically. (Pls. Nos. XIV–XVI picture their examination in the laboratory of the Tokyo Metropolitan Police Board.) In this latter place from 3,000 to 4,,000 rodents are examined daily even in ordinary times, but when an epidemic occurs the number is increased to an enormous figure. As plague outbreaks in Japan are usually preceded by rat infection, these examinations are to be regarded somewhat in the light of a reconnoissance. The utility of this precaution is illustrated by the fact that in 1904 an infected rat was discovered in Yokohama before any human victim had been found and as a result the source of the infection was sought and finally traced to a British steamer, the warning being given in time. During epidemics, the examination of rats is even more necessary, for it is only by such a process that the condition and the manner of propagation of the pestilence are actually known.

To judge from the experience of the past, it can be suggested that in examining rats particular attention should be paid to their submaxillary and cervical glands and to the spleen. These organs in most cases show the evidence of infection, if there be any. The inference to be drawn is that the rat receives the plague germ through the mucous membrane of the mouth and throat.

An important work during an epidemic is the bacteriological examination of specimens obtained from the patients and the dead, for the diagnosis must depend upon both clinical and bacteriological observation. In suspicious cases, material for investigation is taken from the affected parts, such as the glands or skin. The sputum of the patient is also frequently subjected to examination. In the dead, the heart's blood and spleen and the contents of the glands or lungs are scrutinized. Microscopical examinations are undertaken, cultures are made and animal experiments are performed with the specimens which are gathered. Agglutination by a pest serum, of the bacteria obtained is also studied. Obviously it is often difficult to assure one's self of the discovery of an infection by any of these means, but there are many cases on record where suspicious substances such as cotton, filth, and rotting grain harbored the *Bacillus pestis;* as an illustration I will cite the fact that in 1899, in Osaka, cotton waste was found to contain the plague organism.

The searching for patients, especially when the first invasion of plague is suspected, is also an important portion of the preventive work. Physicians are expected by law to report any infectious disease which they discover, but such reports are in most cases not reliable; indeed, during

an epidemic period this source of information reveals only about one-tenth of the whole number of cases actually existing. In the localities which have never before been visited by plague, the physicians themselves are ignorant of the nature of the disease and they may thus permit an outbreak to spread rapidly. This unfortunate condition was illustrated in Kagawa during the past year.

As a means of promptly seeking out patients in places which are frequently in danger of an outbreak of pest, the physicians attached to the police are given authority to examine the bodies of all persons dead from acute, febrile diseases. During epidemic periods such examinations give results parallel to those obtained by the investigation of the rats which are caught, and that such precautions can not be neglected is shown by the experience obtained in the past. The following table gives the number of plague patients discovered during the outbreak in Osaka and Kobe during the past year:

	Osaka.	Kobe.
Physicians' reports	49	30
Inspection of dead	27	26
Inspection of houses	34	33
Among the isolated suspects	20	0
Miscellaneous	4	1
	134	90

The value of the inspection of the dead is apparent, for the foregoing table renders it evident that in Osaka 27 and in Kobe 26 instances would have occurred in which bodies which really were infected with plague would have been regarded as being dead of other diseases and thus the number of foci of the epidemic would have been increased.

The inspection of apparently healthy persons living in an infected locality or its vicinity is not an easy task, though a very necessary one. Epidemics are most liable to prevail in places where ignorant people reside and this fact alone greatly hinders the work of the health inspectors. Only by reason of their devotion to duty and work can their efforts be successful. The value of their work was illustrated during the outbreak of the second epidemic in Tokyo. In the latter city, with the exception of three early cases out of a total of 13, a suggestion and warning was given by the diagnosis of plague in persons apparently healthy and these persons were removed and isolated. During the present epidemic in Osaka not a few of the dangerous patients were discovered by the general inspection of healthy persons. However, the task of finding plague cases by such a method becomes more difficult in proportion as the epidemic spreads and the patients are found scattered over a wider area. As aforesaid, plague in a patient must frequently be diagnosed by both a clinical and a bacteriological examination and the application of such tests must be undertaken as speedily as circumstances will permit. The absolute diagnosis is usually effected within

forty-eight hours after the finding of the suspicious patient or of the dead body and during the examination the patient or the dead body is to be regarded as if it were a genuine instance of plague infection.

Even during ordinary times, the killing of rats is enforced in localities where imported freight is stored or where laborers live and this enforcement is made general when there is danger of an outbreak of plague. At least two days before the general sanitary precautions outlined above are applied, rat catching and killing devices, such as traps and poisons are distributed to every dwelling and warehouse. Formalin vapor or sulphur dioxide is used in buildings which can be tightly closed. All localities which are constructed so as to permit of the abode of rats are rebuilt, the sewage is improved, and all filth is burned. The above constitute the measures employed for removing and killing rats. The occupants of the dwellings in which infected rats are frequently found, or in which plague patients are discovered, are removed and the whole house disinfected and rendered unfit for rodents by the sanitary measures adopted in the killing of rats; in order more effectually to destroy the latter, galvanized-iron walls are erected around such houses and all exits of the sewers are closed by means of metal nets. If the infected area is extensive it is divided into small sections, and the same method is applied to each one of these. The first steps in sanitation after these precautions have been taken are to search for and destroy the rats, to disinfect the furniture and finally the entire building. For the latter purpose carbolic acid, corrosive sublimate and lime are used, articles of an especially suspicious nature being at times steamed, boiled, or burned; all of the disinfected objects are ultimately exposed to the sunlight. In case the building is situated in such a manner as to render the application of sanitary measures difficult, it is sometimes desirable to disinfect by burning the entire edifice. Such extreme measures I believe to be especially justifiable where the infection has not as yet spread to a large area, and they are not infrequently made use of in Japan. The galvanized-iron fence or wall which is erected before fire is set to the premises prevents the rats from finding their way to the neighboring houses, and thus possibly spreading the disease.

However, the difficulties attendant upon the entire destruction of houses for purposes of disinfection are in some instances insurmountable, and this is particularly the case when plague patients and infected rats are scattered over a large area. The present epidemic in Osaka and Kobe illustrates such a situation. If extreme measures were to be applied to these cities practically whole districts would have to be burned.

As has been remarked above, the zinc fences prevent the escape of rats during the work of disinfection and their effectiveness in segregating an entire area before an epidemic becomes general has been abundantly illustrated during the outbreaks in Yokohama, Kobe, and Tokyo. These walls present a most peculiar appearance. (See Pls. X–XII.) Their

height varies according to the circumstances, but usually it is about 3 feet with a foot or two buried in the ground. Rats can neither climb such a wall nor burrow under it. The use of zinc for such a purpose is apparent, for it is not liable to rust as are other metals, and it may be used repeatedly. These walls have been utilized during every epidemic, the largest one being constructed in Tokyo in 1903. In this instance the area inclosed was three-fourths of a mile square, with partitions dividing it into several sections. In addition, every other place where infected rats were found was inclosed within other fences and the total length of the walls so used measured 29,148 feet, or about 4 miles.

The discovery of a case suspected of having plague is promptly followed by the proper measures to prevent further spreading of the disease. Inspecting physicians hurry to the patient's residence and, if he really proves to be attacked by the pest, the victim is at once conveyed either to a hospital for infectious diseases or to one of isolation where proper treatment can be given. Should a dead body be discovered infected with the plague bacillus, the cadaver is first disinfected externally and then cremated. Each member of the family and the contacts in the neighborhood are then sent to an isolated detention dormitory where they are given a daily examination during their term of quarantine. They are subjected to all possible means of disinfection and prophylaxis, including the conferring upon them of passive immunity by inoculation with pest serum, whereas those from the neighborhood who are in less danger of having contracted the disease are given pest vaccine. In every epidemic this serum and vaccine have been generally used, over 10,000 individuals having been inoculated, but we have been unable to obtain exact statistics as to the value of these remedies during the epidemics on the main Japanese islands.

However, during the outbreak in Tainan, Formosa, occurring in 1901, the conditions were such that we were able to obtain valuable statistics and the large number of persons which were inoculated with pest vaccine gave suggestions as to the value of vaccination. Of 10,876 persons inoculated in Tainan only seven were attacked by plague; while out of about 40,000 persons who failed to receive this treatment, more than 500 were infected. From such statistics it is not difficult to believe in the favorable results of vaccination, although we are not yet in a position definitely to determine its value.

Those plague commissioners and officials who from the nature of their duty frequently came in contact with the patients, have received prophylactic inoculations of the serum (16–20 cubic centimeters). Not one of these individuals has as yet been infected. Instances are many in which the value of the serum as a preventive has apparently been demonstrated.

For the treatment of plague patients two methods may be recommended—one the extirpation of the buboes and the other the inoculation

with serum. The efficiency of the extirpation and of the serum treatment depend on the stage of plague development which exists at the time they are instituted; when performed in an early stage, a favorable result may be expected, but in a later one these measures become futile. As a consequence, it is important to obtain a positive diagnosis as early as possible.

That good results may be obtained from serum treatment in such cases admits of no dispute. During the first outbreak in Osaka, Yersin's serum was used, but owing to the scarcity of the supply of this remedy, the results fell short of our expectations. Since 1900 our institute has been preparing the serum to meet with the constant demand. For the patients actually suffering from the disease, comparatively large quantities (200–240 cubic centimeters) are required for inoculation, and although we are not in a position to ascribe to the pest serum a value as absolute as to the diphtheria serum, there is no doubt of the efficacy of the former remedy. The following facts demonstrate this:

A series of experiments was conducted in the Tainan isolation hospital (Formosa) with the view of comparing the results of the serum with those of an early extirpation of the buboes and general systematic treatment. Of the 56 patients treated by the latter method, 35 (62.5 per cent) died of plague, while out of the same number inoculated with serum the death rate was only 33.9 per cent. From these experiments it is seen that the use of the serum reduced the death rate by about one-half. Our experience during the epidemics in Japan has shown that the most effective treatment is that in which both serum inoculation and extirpation of the buboes are performed in as early a stage of the disease as possible.

RATS AS THE PROPAGATORS OF PLAGUE.

The fact that rodents are always closely connected with an outbreak of plague (in all times and places) admits of no dispute. The epidemics in Japan have invariably been traced to rats.

Obviously these animals have a high susceptibility to the pest infection; their habits also constantly bring them in contact with filth in which the plague germ is present, besides which they feed upon one another. These facts must favor the spread of plague. The finding of human victims of the pestilence is almost invariably preceded by the discovery of plague-infected rats. Hence the killing of rats must be resorted to as the first and most important step in the prevention of an epidemic. In the first outbreak in Osaka and Kobe the pestilence was gradually stopped by an urgent effort directed at destroying these rodents; and the ones in Tokyo and Yokohama were confined to a small area by the strict enforcement of these same measures. The number of rats killed in Tokyo since 1900 and up to the present time amounts to the enormous figure of 4,820,000—that is to say, the average is more than 800,000 a year. In other words, if these dead rats were laid side by side they would extend for a distance of

over 75 miles. The price paid by the local government for these animals, which were bought from the people, in Tokyo alone has amounted to 160,000 yen.

However, the extermination of rats is complicated by the fact that the rodent increases at an enormous rate; as a rule within one month of pregnancy the female gives birth to five young ones at a litter, and the young reach puberty and become pregnant at the age of three months; thus these animals multiply in a geometric progression. Furthermore, if rats are destroyed by artificial means, such destruction only lessens the struggle for existence and then the rate of multiplication is much increased. In Tokyo more than 4,800,000 rats have been killed; yet we can hardly notice any considerable decrease in the number of these animals.

In Japan, for killing rats, poisons such as arsenic and phosphorus, together with traps, are chiefly used. The typhoid bacillus of the rat, which has been effectively used for killing field mice, has been found useless for house rats and therefore we no longer employ it.

Someone has offered the following suggestion for the disposal of the usual breed of household rats (*Mus rattus*), which unfortunately is the species most liable to be attacked by plague. This suggestion is to introduce *M. decumanus*, which is a persistent enemy of *M. rattus*, and which is comparatively more resistant to the infection. Such a suggestion appears to me impracticable for from my actual observation it is evident that so far as the epidemics in Japan are concerned, the *kind* of rat has been of very little importance in propagating plague. Moreover, the results of biological researches tend to confirm the fact that although two distinct species of rats are found, the one most prevalent in Japan is a race which is a mixture of the two and which is also susceptible to plague. Such a fact rather contradicts the assumption that the two species are natural enemies to each other. Therefore, the above recommendation can hardly be taken as a basis for preventive measures against plague.

The best way to destroy rats in connection with the restriction of pest is to prevent their abiding in or at least to expel them from human habitations. In order to accomplish this purpose I suggest that all buildings be rebuilt according to a plan which will serve this end. This particularly applies to Japanese houses, because in most cases the latter are built of wood, and such buildings are liable to provide quarters for rats. I understand that the United States is planning a reconstruction of "Chinatown" in San Francisco. I highly approve of such a step in view of the situation of that port, which is constantly threatened with a plague invasion. In Manila, also, the cause of the recent fortunate decline of the plague epidemic, I believe, can chiefly be attributed to the rebuilding of the city since the Islands became a possession of the United States.

In Japan, the insular territory of Formosa which has been invaded by plague is now being surveyed by the sanitary officials, and the buildings in the central part of Tainan City have already been reconstructed so as to keep the rodents out. As a consequence, the ravages of the pestilence are now practically confined to the villages or to groups of unsanitary habitations of the natives, who live with the rats and permit these animals to flourish.

The following table demonstrates that during a plague epidemic the number of infected rats runs parallel with that of the patients discovered:

	Osaka.		Kobe.	
	Patients.	Infected rats.	Patients.	Infected rats.
May	1	1	0	2
June	0	0	0	0
July	0	0	0	0
August	0	0	2	3
September	0	5	8	11
October	6	39	4	17
November	45	119	36	151
December	82	634	40	405

The number of rats examined during the past year in Osaka amounted to 1,195,116. Of these, 19 infected animals were found previous to May, making a total of 817 in all. In Kobe 553,616 rats were examined; the number of infected ones can be ascertained from the table. From this it may be inferred that the prevalence of the human infection bears a direct proportion to the number of infected rats found, and that the extent of the epidemic may be known approximately by the area of the localities in which infected rats are encountered.

Our attention is particularly directed to the fact that the fiercest ravages in every epidemic are evidenced in winter rather than in summer. This may be due to the resistance which the pest bacillus has to cold, although it appears that this point needs further consideration. A particularly interesting fact is that in both Osaka and Kobe the number of infected rodents found during the last two months of 1905 was very large as compared with the number of plague patients, and also the statistics up to the middle of January of this year show a remarkable feature in that there were 240 infected rats against three patients in Kobe, and 179 against nine patients in Osaka.

During the present epidemic I have had opportunity to make close observations as to the prevailing conditions, and have discovered an interesting fact concerning the habits of the rodent. Rats generally live on the ceiling below the roof, but in winter they change their abode by removing to the ground just below the floor. Therefore, in order to discover the openings which harbor the rodents, the ground should be dug to a depth of 1 or 2 feet, the rat-holes are thus exposed and the animals can be exterminated. This method, owing to the subterranean habit of rats in winter, invariably secures a great number. These animals are

gregarious and consequently, if one of their number becomes infected in the winter quarters, then many must inevitably fall victims to the disease, but obviously there is less danger from rodents which live underground and apart from human habitations than there is from those dwelling in the houses themselves. It is for this reason that during winter epidemics a very large number of infected rats, as compared with human patients, is encountered.

The weak rodents are constantly menaced and frightened by the strong ones, so that the individuals which have been attaked by plague must maintain constant vigilance so as to be able to flee from their pursuers. As a weak rat has not sufficient strength to climb a fence or to reach any considerable height it is obliged to wander on the surface of the ground, thus providing for the spread of plague.

The large number of children who are infected by playing on the ground would seem to emphasize the fact that in this situation the bacillus is most readily encountered. The following table shows the number of children infected with plague during the winter epidemics:

Number of patients.	1900, Osaka.	1905.	
		Kobe.	Osaka.
Total patients	144	90	134
Patients under 15 years	56	36	51
Percentage	38.8	40.0	38.06

From the above it will be seen that the number of patients under 15 years of age is more than one-third of the total. (See also Pl. II.) The subterranean and gregarious habits of rats, the sowing of the plague germ on the ground, and the large numbers of young patients—all of these are closely connected with winter epidemics.

It is apparent that measures for killing rats must of necessity involve the application of biological knowledge bearing on the habits of these animals, but our knowledge in this respect has yet to be perfected. The methods of destruction used in Japan to-day consist in the use of poison or of traps. Such means have only a temporary effect and the results of their use can not be considered to be of sufficient permanence to root out the evil. The destruction of rabbits in Australia was undertaken in such a way as to produce a permanent effect and we have much to learn from the method involved. It was based on the fact that the rabbit is a polygamous animal and therefore if as many females as possible could be destroyed by artificial means, the end would be a struggle among the males for their possession. The result of such a practice, of course, can not be expected in a brief period, nevertheless it is of a permanent character. I suggest that something of this nature be planned for the destruction of the rodents.

CONCLUSION.

In conclusion I desire to suggest what I consider to be an ideal plan for combating plague. I believe that the fatal pestilence, however obstinate its ravages and terrible its effects, could be fought and vanquished by the persistent efforts of man and I also believe that where human endeavor, backed by money, is determinedly directed against it, it must yield. But the efforts, however laborious, the money, however vast, can be of no value unless they be accompanied by the application of scientific knowledge. The danger of plague invasion through open ports must increase in proportion as international commerce progresses and maritime enterprise advances. Again, where man has fixed his abode the rodents accompany him to share it; and the unwelcome creature becomes the cause of the dreadful evil. In ports which vessels from infected regions frequent, an epidemic of the pestilence may not be difficult to exterminate. But if infection after infection is repeated from fresh importations the expenditure of large sums of money and much tedious effort become involved. In regions like India and southern China, plague appears to be deeply rooted, prevailing almost incessantly for several years and during each year attacking more than 200,000 victims. It is apparent that we can not avoid the danger of invasion by the pestilence at any moment, so long as we do not cease intercourse with these regions. To be content with the mere placing of quarantine on the incoming vessels from these places or of enforcing rat killing and other sanitary measures in the open ports seems to me to be but imperfect protection. Why not extend the combat to the source of danger and destroy the cause of the evil permanently? Plague is not only objectionable to the people of one locality but it is an enemy of mankind in general. All civilized nations must fight this common enemy. I believe there should be an international conference to discuss a plan to collect money and to organize an international army to combat and vanquish this disease wherever it appears. Expeditions should be sent to the interior regions of India and south China. The cost of such an enterprise would be only a small part of what the civilized nations are constantly expending in keeping armies and navies. Even the amount which every country is spending for the prevention of the pestilence would suffice. My suggestion only lacks a leader, and I see that the United States, one of the greatest nations of the earth, has such a leader in the person of its President, Theodore Roosevelt, who has already done so much for humanity and whose noble works are being admired by the whole world.

ILLUSTRATIONS.

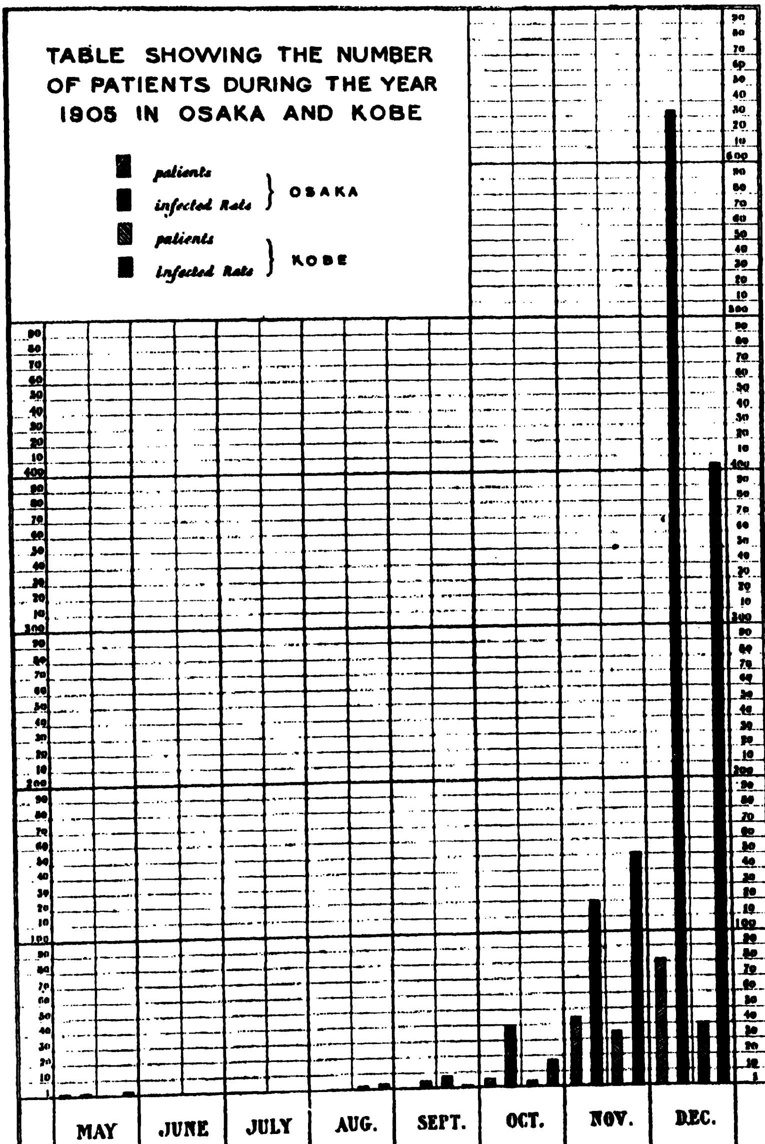


TABLE SHOWING THE NUMBER OF PATIENTS DURING THE YEAR 1905 IN OSAKA AND KOBE
patients
infected Rats
OSAKA
patients
Infected Rats
KOBE
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.

PLATE I.

DIAGRAM SHOWING THE RATIO OF MORTALITY WITH REGARD TO THE AGES OF THE PATIENTS

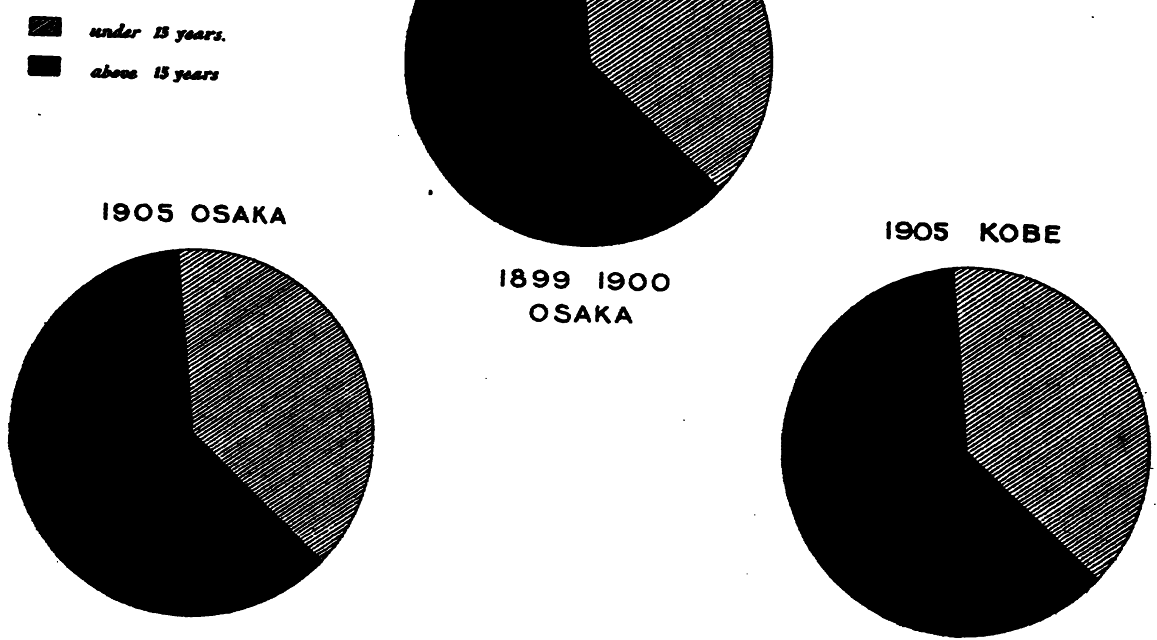


PLATE II.

PLATE III.

PLATE IV.

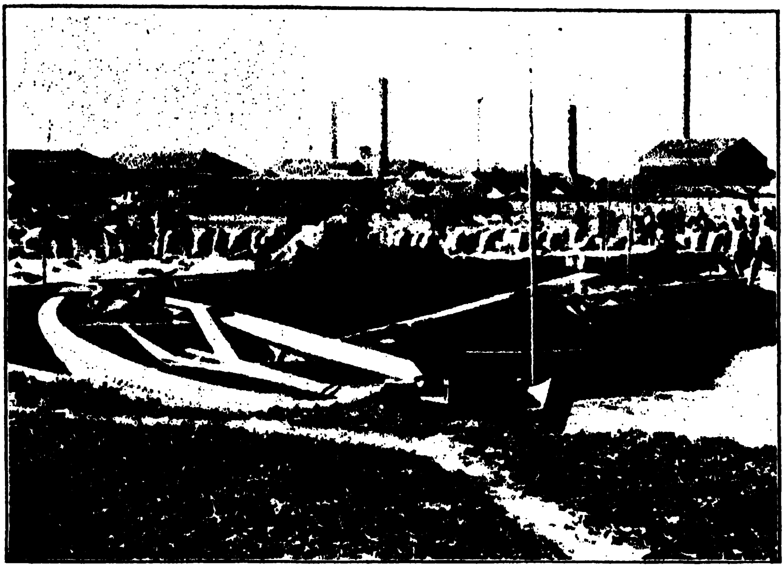


PLATE V.

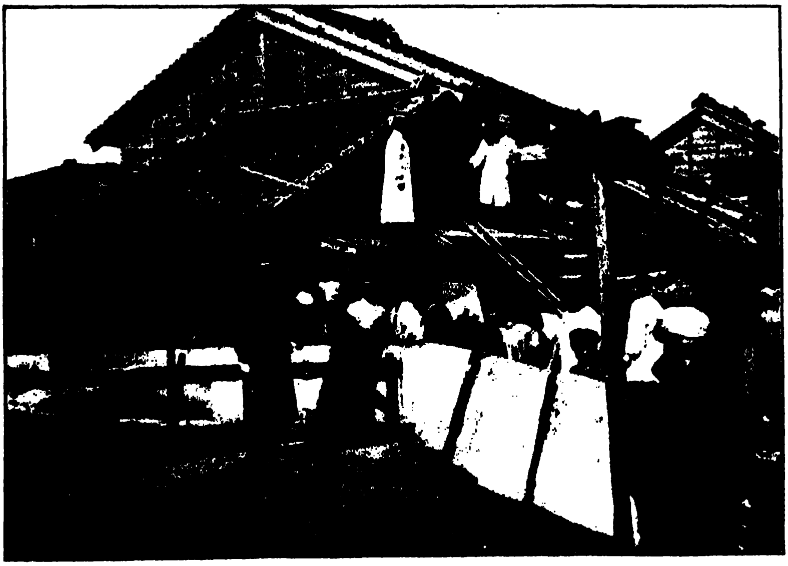


PLATE VI.

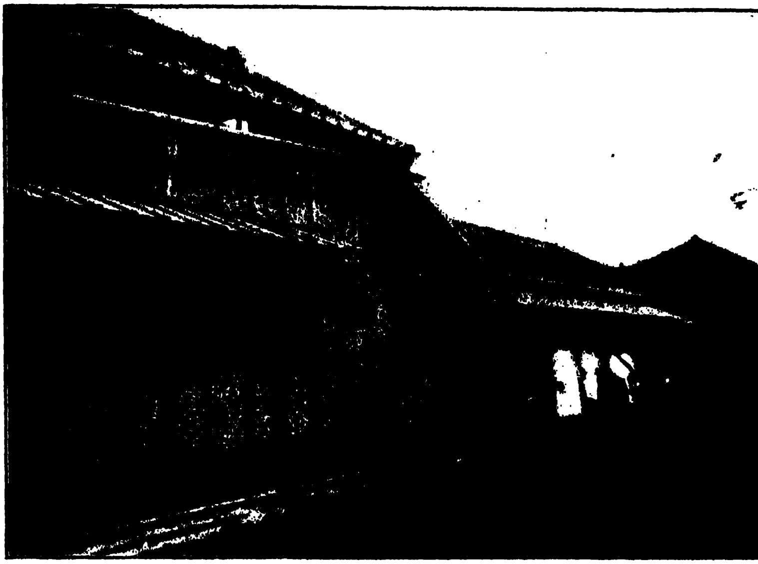


PLATE VII.

PLATE VIII.

PLATE IX.

PLATE X.

PLATE XI.

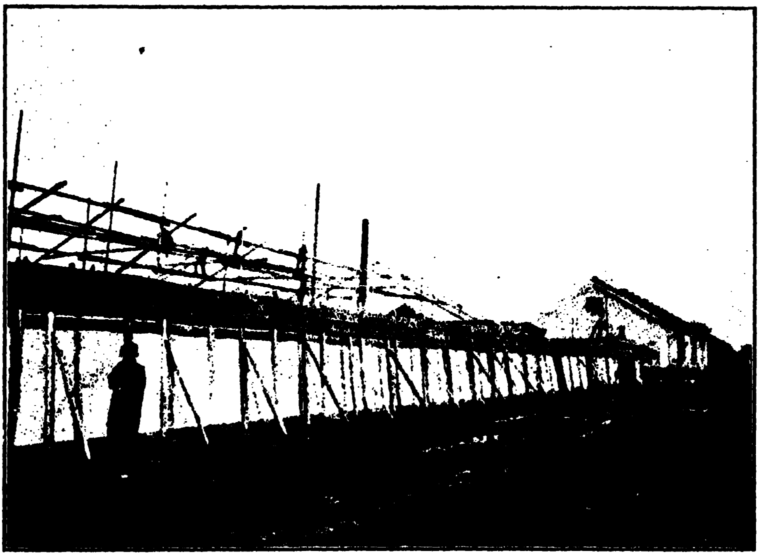


PLATE XII.

PLATE XIII.

PLATE XIV.

PLATE XV.

PLATE XVI.

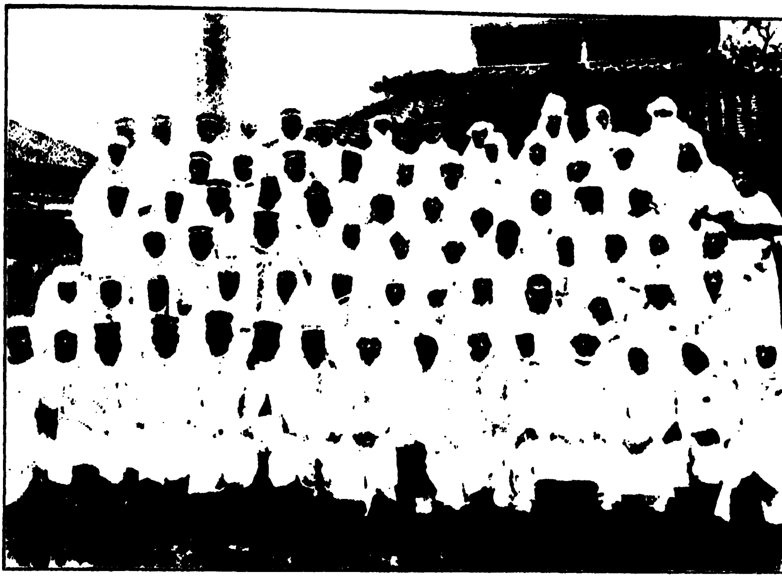


PLATE XVII.

PLATE XVIII.

PLATE XIX.

PLATE XX.

PLATE XXI.

OBSERVATIONS ON THE EPIDEMIOLOGY OF DYSENTERY IN JAPAN.[1]

By K. Shiga.

(From the Imperial Institute for Infectious Diseases, Tokyo, Japan.)

Before discussing my own work on dysentery I consider it to be advisable to give a brief outline of the course of the epidemics of dysentery in Japan, for I believe that in no land other than our own has this disease taken such deep root or appeared with such destructive results. Dysentery for a long time has been prevalent in Japan, its beginning probably antedating the present time by hundreds of years, but reliable statistics are only available during the last three decades.

The Japanese Empire extends from 21° 48′ to 50° 56′ north latitude, and following the geographical configuration the country is divided into seven provinces which, as a rule, are used as the units in statistical work. Therefore the southern portion of Japan is tropical or subtropical, whereas the northern part belongs to the cold zone. Amœbic dysentery occurs in Formosa, but, up to the present time, epidemic or bacillary dysentery has not with certainty been proven to exist in that island; in the island group at the extreme north (*Chishima*) dysentery has not been observed at all. The mainland of Japan, on the other hand, is in the temperate zone, and it is exactly in this region that epidemic dysentery appears; and therefore, for the purposes of this paper, only the mainland needs to be considered. This consists of four islands—Kiushu, Shikoku, Honshu, and Hokkaido; Honshu is again divided into the western, central, and northern provinces. The following table gives the degrees of latitude occupied by the regions in question:

Kiushu: From about 31° to 33° 58′ north latitude.
Skikoku: From about 32° 42′ to 34° 34′ north latitude.
Honshu: Western province from about 33° 30′ to 35° 40′ north latitude.
Central province from about 34° to 37° north latitude.
Northern province from about 37° to 41° 38′ north latitude.
Hokkaido: From about 41° 21′ to 45° 30′ north latitude.

If we study the course of the epidemics of dysentery in these six divisions of Japan we encounter a very interesting picture. In the past

[1] Read before the third annual meeting of the Philippine Islands Medical Association at Manila, March 3, 1906. Translated from the German by P. C. F.

thirty years two extensive outbreaks have traversed Japan from the south to the north, the first, and lesser one, beginning in Kiushu in the interval between 1883 and 1885. It was only in 1885 that it extended into Shjkoku and in the next year (1886) into the western parts of Honshu, from which latter portion of the country it soon disappeared, not reaching farther toward the north. The second and larger one, in its movement, can be described very graphically. Between 1892 and 1894 a great epidemic of the disease again appeared in Kiushu, the number of the sick reaching nearly 45,000 to 50,000; in 1895 it suddenly lessened so that the patients decreased to about 14,000, and in the next year they further diminished to about 6,000. Meanwhile, in 1893 the epidemic pushed forward into Shikoku, reaching its maximum in 1894, the number affected being 34,600, and from this point it extended into Honshu, however, only being observed in the western provinces of this district in 1895—that is, two years after its appearance in Shikoku. In 1896 the disease extended to the central province, and during three years it ravaged that district with frightful intensity. Just as it was beginning to diminish samewhat in this latter region it suddenly appeared in the western provinces, in which portion of Japan it reached its highest point, so that there the number of cases reached 60,700 in 1899. Up to the present time this great epidemic has never appeared with any degree of intensity in Hokkaido, the northernmost of the large islands of the Empire, probably because of the cold climate—for in that portion of the country we have only encountered some hundreds of cases in one year.

As has been seen, the great waves of the epidemic of dysentery gradually rolled from the south toward the north, the greatest amplitude in one and the same region remaining during one to three years. After reaching its maximum, it suddenly diminished, to repeat itself, as statistics show, at intervals of from ten to twenty years. This interesting phenomenon can probably be explained through the immunity to dysentery which persists for a certain time after infection with the disease. About thirty years ago (1879) an extensive epidemic of dysentery appeared in the northern provinces of Honshu although, at about this time, only a few cases were observed in other portions of Japan. I regard this outbreak in the north as the termination of a great one which probably also traveled from the south to the north and which represented the ultimate extensions of the epidemic. Owing to these considerations I believe the conclusion to be justified that great epidemics of dysentery will threaten us every ten to twenty years if we can not limit them by modern methods of prophylaxis. A detailed study of the prevalence of dysentery in individual localities strengthens this assumption.

Upon the appearance of dysentery in small cities or villages, almost all susceptible persons contract the disease, so that, in Japan, it is not unusual to encounter 5 to 10 per cent of the entire population of a village

attacked by the malady; but in this event, no cases, or at least very few, appear during the next following year, the epidemic entering the neighboring hamlets. If, on the other hand, dysentery, during the first year, appears only in the late autumn or early winter and if only a small number of people are attacked, then in the coming season a large epidemic, which, as a rule, will entirely disappear in the third year, must be expected. Reinfection, by which the disease is contracted in two successive years, is, according to my experience, of very rare occurrence. Among 10,000 patients sick with dysentery I have only observed such a recurrence in three or four instances and in these the first attack had been a very mild one. I have never observed a reinfection with patients in whom the course of the disease had been very severe.

The main factors in the spread of dysentery are the hosts (i. e., the individuals who convey the bacillus in the intestinal tract); and of these, three classes are to be considered: First, healthy persons; second, those who have mild cases of dysentery or of diarrhœa respectively; third, convalescent dysentery patients.

As is the case in typhoid, cholera, or diphtheria, perfectly healthy individuals may serve as hosts for the bacillus without manifesting a single symptom of dysentery, but to bring the proof of the presence of the bacillus in healthy stools is extremely difficult; however, Conradi succeeded in so doing in three cases which he encountered in a small epidemic of dysentery at Metz. During an outbreak of dysentery we often encounter patients affected with this disease or with diarrhœa, who clinically only show the symptoms of a simple intestinal catarrh but in whom dysentery can with certainty be demonstrated by bacteriologic methods and such cases appear not only in times of epidemics but also during the cold seasons. So-called winter diarrhœa often belongs to this category and in this way the bacillus is conveyed from person to person. I do not believe the organism can live for any length of time outside the human body. It maintains its existence therein throughout the winter and if it reaches the outside world during a favorable time in the early summer it may then cause a great epidemic. We were able to demonstrate the presence of the dysentery bacillus in stools which macroscopically had a perfectly normal appearance and which had been taken from patients one or two weeks after their convalescence. The fact that dysentery patients who were dismissed from the hospital as entirely cured would at times suffer a relapse after several weeks, or that the disease appeared in their respective families, would seem to prove that the bacillus can be present in the stools of convalescents for even a longer time than that given above; however, the question as to whether the specific organism can remain in the stools of convalescents for years, as is the case with typhoid, is still an open one.

Forster has lately emphasized the fact that the bacillus of typhoid finds a permanent habitat in the gall bladder, from which, from time to time,

together with the bile, it finds its way into the intestinal canal. Such an occurrence we have not as yet been able to prove in the case of dysentery. Dr. Amako in Kobe, made cultures from the bile and spleen of 16 cadavers dead of dysentery, but he never was able to demonstrate the presence of the bacillus and these experiments made the assumption probable that the organism of dysentery, unlike that of typhoid, can not find its way into the circulation.

TYPES OF DYSENTERY BACILLI.

The etiological importance of the dysentery bacillus as the causative factor in dysentery was first proven by Flexner[2] and by Strong[2, 3] in Manila, and the latter author, at that time, had already strictly distinguished between two types of dysentery—i. e., bacillary and amœbic. The latter type, as a rule, takes a chronic course and amœbæ, generally, can at all times be discovered in the stools, whereas the former is an acute disease the causative factor of which lies in the bacillus of dysentery.

Kruse,[4] in 1901, first called attention to the fact that an organism discovered by him in Germany is not entirely identical with the one observed in Manila. During an epidemic of dysentery occurring in a prison he encountered a second bacterium which differed from the Shiga-Kruse bacillus only in respect to its serum reaction; the latter organism he termed the pseudodysentery bacillus, and this variety was soon thereafter also discovered by Spronck[5] in Holland. Martini and Lenz[6] confirmed this observation of Kruse and even extended it, for Lenz[7] succeeded in differentiating the Shiga-Kruse stem from the Manila one by cultural methods, in that he demonstrated that the former did not, whereas the latter did, ferment mannite which he had placed in his culture media. Hiss and Russell,[8] independently of Lenz, but at the same time, showed that a bacillus, isolated by them from a case of infantile diarrhœa, and termed "bacillus Y," differed from the Shiga-Kruse stem in its fermentive action on mannite, and following this publication a further paper by Hiss[9] appeared in which the author separated the bacillus of dysentery, which he had isolated from patients attacked by dysentery and infantile diarrhœa, into three groups based upon cultures obtained on media containing, respectively, mannite, dextrose, maltose, saccharose, and dextrin. Gay[10] distinguished two groups of dysentery bacilli, founding his deductions on the bacteriolytic action of and the protection given by the immune serum, and finally Hiss,[11] using the fermentative action on the above-mentioned carbohydrates, succeeded in giving a reliable classification; and

[2] *Phil. Med. Journ.* (1900), Sept. 1, **6**, 414. Also *Bulletin of Johns Hopkins Hospital* (1900), Oct., **11**, 231.

[3] *Journ. Am. Med. Assoc.* (1900), Aug. 25, **35**, 498 and 501, also *Circulars on Tropical Diseases* (1901), No 1, Feb., Manila, P. I.

[4] *Deut. Med. Woch.* (1901), **27**, 370, 386, 637.

[5] *Baumgartner's Jahresbericht* (1901), **17**, 473.

[6] *Zeit. für Hyg. u. Infect.* (1902), **41**, 540.

[7] *Loc. cit.*, 540.

[8] *N. Y. Med. News* (1903), Feb. 14, **82**, 289.

[9] *Journ. Med. Research* (1904), new series, **8**, 12.

[10] *Univ. of Penn. Med. Bull.* (1903), **16**, 171.

[11] *Loc. cit.*, p. 1.

this action also accords with the agglutination phenomena and the absorbability of the agglutines. The following table gives his conclusions:

Group 1: **Ferments dextrose only (Shiga, Kruse, Flexner's New Haven bacillus).**

Group 2: **Ferments dextrose and mannite (bacillus Y, Ferran, Seal Harbor bacillus).**

Group 3: **Ferments dextrose, mannite, and saccharose (Flexner-Strong Manila bacillus).**

Group 4: **Ferments dextrose, mannite, saccharose, maltose, and dextrin (Harris, Gay, Baltimore and Wollstein's bacillus).**

The author, in conjunction with Ohno, has been working in Japan during the past year on the types of the dysentery bacillus. As many stems of this organism as it was possible to procure were sent from various regions and these were accurately investigated. At first we studied the power these organisms possessed of causing fermentation in mannite and other carbohydrates (dextrose, maltose, saccharose, dextrin, and lactose), following the methods laid down by Hiss, but, for the purpose of classification, we also took cognizance of four other properties—i. e., (*a*) indol reaction, (*b*) agglutination, (*c*) bacteriolysis, and (*d*) preventive action of the immune sera. The dysentery bacilli could be divided into three groups, if the indol reaction is to be taken as a criterion, as follows:

Group 1: Nonindol forming (Type I).

Group 2: Forming indol with difficulty (Type II).

Group 3: Forming indol with ease (Types III, IV, and V).

The third of the above groups forms indol with the greatest readiness in a 1 per cent aqueous peptone solution; the reaction appears promptly and very decidedly. The second does not form indol in this solution during one to two weeks, but gives a weak and inconstant reaction after three; however, in a 2 per cent culture a distinct reaction can be observed after the expiration of one week. The first does not form indol under any circumstances.

The fermentative actions of these dysentery bacilli on mannite and other carbohydrates are very complicated and somewhat variable. To judge from these phenomena, Ohno was able to distinguish sixteen varieties, among which were six which did not ferment mannite (so-called *non-acid bacilli*) and ten which did (so-called *acid bacilli*). (See Table II.)

The fermentation of dextrose and mannite is constant, but that of maltose, saccharose, dextrin, and lactose varies considerably both qualitatively and quantitatively, and so great is this variation that it seems to me impossible to give a correct classification based upon this property. Owing to this contingency, five groups, the fermentative properties of which could be distinguished with some certainty, were selected from the numerous subvarieties. (See Table III.) The first four groups in this classification accord entirely with those of Hiss, but in addition to these a fifth, which has not been differentiated by Hiss and other observers, has

been added. This type can be distinguished from the fourth one in that after twenty-four hours, it gives an acid reaction on a culture medium containing mannite but this gradually disappears until finally, after four days, the medium becomes alkaline and remains so. Therefore this type is a form intermediary between the acid and non-acid dysentery bacilli.

The agglutination phenomena of these five groups of dysentery bacilli on the whole agree with the fermentative action on mannite and the other carbohydrates. The rabbit yields a specific immune-serum against the different types of the organism, and therefore this animal is recommended for carrying on the experimental work; the goat, on the other hand, frequently does not give a specific immune-serum, and hence can not be employed to so great an advantage; and finally, the immune-serum to be obtained from the horse is useless for this purpose, as it generally gives an unreliable group reaction. Types I and V form the two end points in the agglutination phenomena; each immune-serum obtained by their use has a specific but no common reaction, or, in other words, neither one has agglutino-receptors common to the other. Types II, III, and IV show a gradual transition from one extreme to the other. (See Table IV.) The bacteriolysis brought about by the rabbit immune-serum is also not a strictly specific phenomenon, because the serum of one group frequently exerts more or less of an action on the others.

If all of the experiments outlined above are taken into consideration, it finally becomes evident that the total results of the fermentation of carbohydrates, the phenomena of agglutination and those of bacteriolysis are not only not entirely in accord but that they even are somewhat contradictory. Therefore, from the data at hand it seems to me impossible to arrive at a final classification, and consequently, lacking a better one, I will, for the present, retain the division into five groups which I have outlined; a discussion of another system arising from the standpoint of serum therapy will be entered into below.

THE OCCURRENCE OF SPECIAL TYPES OF DYSENTERY BACILLI ENCOUNTERED IN DIFFERENT EPIDEMICS.

As has been shown above, other varieties of dysentery bacilli (*para* dysentery bacilli) exist in addition to the one which was first discovered, and these forms, as well as the one originally described, are etiological factors in the causation of the disease, so that the important question as to the relationship between the first and the subsequent varieties was at once forced upon us. In order to find the answer, the occurrence of the various types of dysentery bacilli in their epidemological behavior was studied, to accomplish which end I obtained many cultures of the organisms from various provinces and countries and subjected them all to exact study. The bacillus which was first described by me was encountered in by far the greater percentage of cases among dysentery

patients in Korea, in the Japanese army in Manchuria, among the Russian troops in Port Arthur and the sailors from the ships of the Baltic fleet which had capitulated, but in the epidemic in Tokyo and some of the provinces of Japan the other varieties were met with in much the greater number of patients; indeed, the original bacillus was identified in only a few of the approximately 200 cases which occurred during the outbreak of the past year in Tokyo. Dr. Amako, in the same year, in an epidemic which raged in the city of Kobe, encountered a very interesting experience. In Kobe dysentery first made its appearance in July, at first with a few sporadic cases in different parts of the city, and in these the *varieties* of the original dysentery bacillus were encountered exclusively; however, in the beginning of August very large numbers of infections occurred in the western portions of the municipality and among these the original organism itself was almost the only one met with, and beginning with this time it was detected in dysentery patients in the most diverse districts of the city, so that subsequently, not only the original dysentery bacillus but also its varieties appeared side by side throughout whole sections of the city. After this time the infections with the original bacillus gradually diminished in number until, finally, toward the end of the epidemic the varieties were the only ones which could be observed. (See Table V.)

It is my opinion that the bacillus first described in 1898, the date when my investigations on dysentery were begun, was present in by far the greater number of cases, because, if this were not the case, I would have encountered many difficulties in its study, for at that time I investigated hundreds of cases of dysentery by bacteriological methods and in almost all I isolated from the stools of the patients the dysentery bacillus which I then discussed in the literature, its identification being chiefly by means of the agglutination reaction (for the greater part with rabbit immune serum). My original stem is the non-acid forming one, identical with Kruse's bacillus. This identity was entirely accidental. The bacillus discovered in Manila in 1900 by Flexner and by Strong is *acid forming*, but, on the other hand, the organism which was kindly sent to me from Manila by Dr. Strong in the past year, was *non-acid* forming and therefore it is likewise purely accidental that the acid-forming variety was first discovered in the latter city. In this connection the opportunity should be taken of mentioning cases in which the original bacillus as well as the varieties have been encountered simultaneously. Gay and Duval report three such instances in acute dysentery in adults, Hastings two, and finally Duval and Shorer six in the summer diarrhœa of infants.

Finally, the dysenteries which are caused by the varieties of the bacillus on the whole seem to take a milder course than those occasioned by the original stem, although a tolerably high mortality also occurs with the former, as will be seen from table.

THE PREPARATION OF A CURATIVE SERUM FOR DYSENTERY.

In conclusion, a few experiments on the preparation of a curative serum, based upon work which has extended over more than a year, will be given. In a practical way these experiments are highly important, as the bacilli of dysentery occur in various types which, from the standpoint of methods of immunization, are considerably removed from one another. For technical purposes, were it possible, it would be highly desirable to prepare a universal serum which would be active against all of the dysentery bacilli, by the treatment of animals *with one type of bacillus only*. Unfortunately, this can not be done. We have already seen that the rabbit immune serum from each of the five is to a certain extent specific in its agglutinating and bacteriolytic properties, and it is therefore *a priori* highly improbable that a universal serum could be prepared by immunizing with one type only.

Horses react toward dysentery bacilli very differently from rabbits; thus, for example, the horse immune serum produced with the original type of bacillus is also more or less effective toward the others, but this group reaction is not sufficiently great to be of value for therapeutic purposes. In immunizing horses I have several times had unfortunate, but instructive, experiences. On one occasion, when the resistance of an animal was so high that it could well endure the injection of 25 agar cultures of Types I and II, it was given a similar dosage of Type V. Eight days thereafter it died of rupture of the liver. A second horse received 10 cultures of Type I and II and after fourteen days 15 agar cultures of Type V; thereupon the temperature rapidly rose to 39.4° and then quickly fell to below normal. The animal died from collapse three days after the injection. These observations seem to show that the treatment with Types I and II did not protect against Type V.

After this disgression I will once more return to my principal subject. After I had first shown that the immune serum of the horse, prepared either with the original bacillus or one of the varieties, was not active against all types, I continued the work with the purpose of ascertaining if a universal serum could be obtained by combining the immunizing properties of the original type and one of the varieties. For this purpose I immunized a number of horses with different combinations of dysentery bacilli and then tested the immune serum with the different types.

The method of testing was as follows: Diminishing quantities of the serum were added to the cultures of the dysentery bacilli in five times the fatal doses, and the resulting mixtures injected intraperitoneally into mice. A fixed quantity on an accurately calibrated platinum oese was taken from an incubated twenty-four-hour agar culture, suspended in an accurately measured quantity of normal salt solution so that 1 cubic centimeter contained exactly 2 milligrams of bacilli. The immune serum was added to test tubes in the following quantities: 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, and 0.005 and all of the tubes were filled to 1 cubic centimeter, after which 1 cubic centimeter of the suspension of the bacilli was added; 0.4 of this mixture was then injected intraperitoneally into each of two mice of 12 to 14 grams weight. As one-fifth of the mixture in the tubes had

been injected in each instance, then the serum in the individual doses was as follows: 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, and 0.001. The result was taken after twenty-four hours.

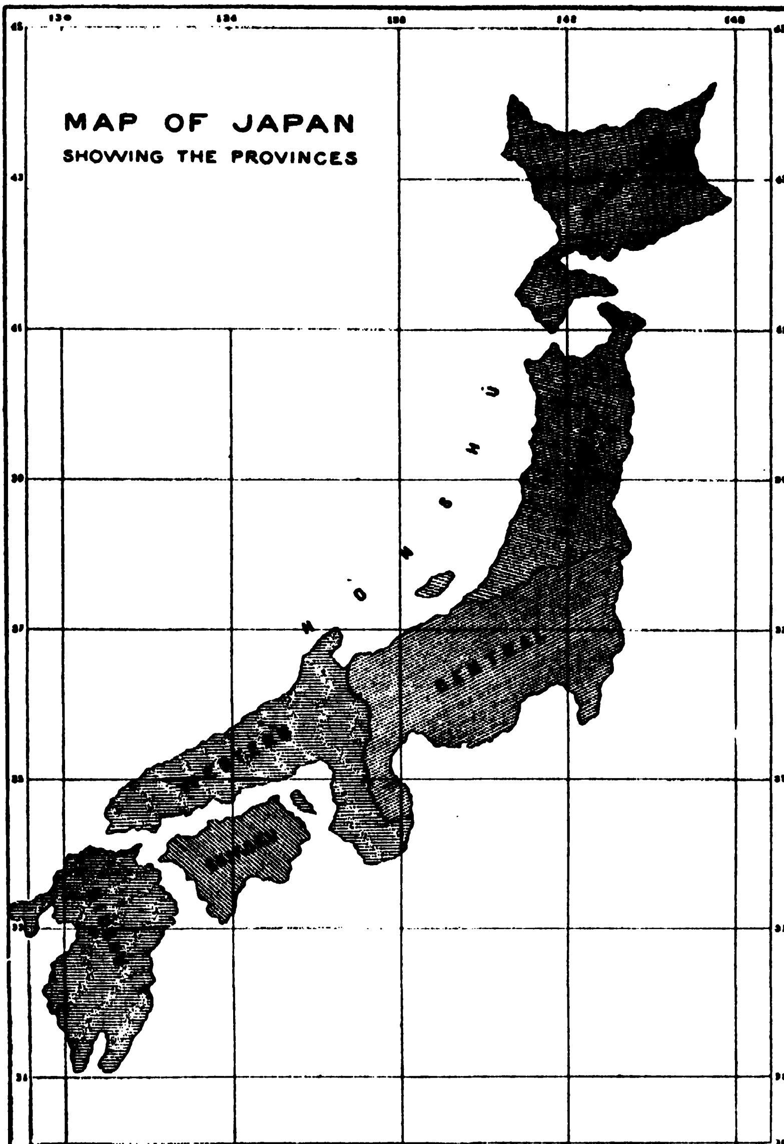
It is necessary to remember that dysentery bacilli very rapidly lose their virulence, so that, after some weeks' standing they are already not appropriate for the purpose of testing sera; however, I have succeeded in maintaining their virulence successfully during more than one year by placing the cultures of the strains in the refrigerator immediately upon their isolation. When they are to be used some of this culture is retransplanted to agar.

A few examples of my numerous experiments with different sera are given below. (See Table VI.) The third type of the bacillus is lacking in this table, as the cultures which I had at my disposition had already lost their virulence and were therefore no longer available for animal experiments, but I have shown in other instances that Type III is acted upon almost as well by serum from Type II as is the latter bacillus itself. As the table demonstrates, the serum from Type I more or less affects Types IV and V, that from Type II feebly acts upon Type I, and is almost without effect upon Types IV and V, but, on the other hand, Types IV and V are closely related to each other, so that a serum from one of these acts almost as well upon the other as it does upon itself.

The above experiments show that a universal serum can be obtained by mixing the immune serum secured from Types I and II with that from I and IV. It is true that if we take three horses and immunize one of them against Type I, the second against Type II, and the third against Type IV, and then mix the sera prepared from all three, we also would obtain a universal serum, but the disadvantages of this method would consist in the fact that the individual titer of each serum would be reduced to one-third of the value which it would have if it alone were present.

For the purpose of immunizing horses the alternating separate injection of cultures of two types is recommended. If a mixture of the two is used, serious suppuration at the point of injection generally takes place, with the result of greatly reducing the acquired immunity. It is also true that as high an immunity is not obtainable if the two types are kept separate but injected simultaneously on both sides of the animal as if they were given in alternate doses, because then the quantity of any one used would only be one-half of the total. It must also be borne in mind that horses are very susceptible toward the original type, whereas they can very well endure the varieties; therefore the immunization can be forced about twice as far with the latter as it can with the former.

Owing to these considerations my advice is as follows: To immunize two horses simultaneously, the one with Types I and II, the other with I and IV, as soon as a high immunity is reached, serum should be taken from both and mixed in equal quantities. By this means the best universal serum can be obtained.


MAP OF JAPAN
SHOWING THE PROVINCES

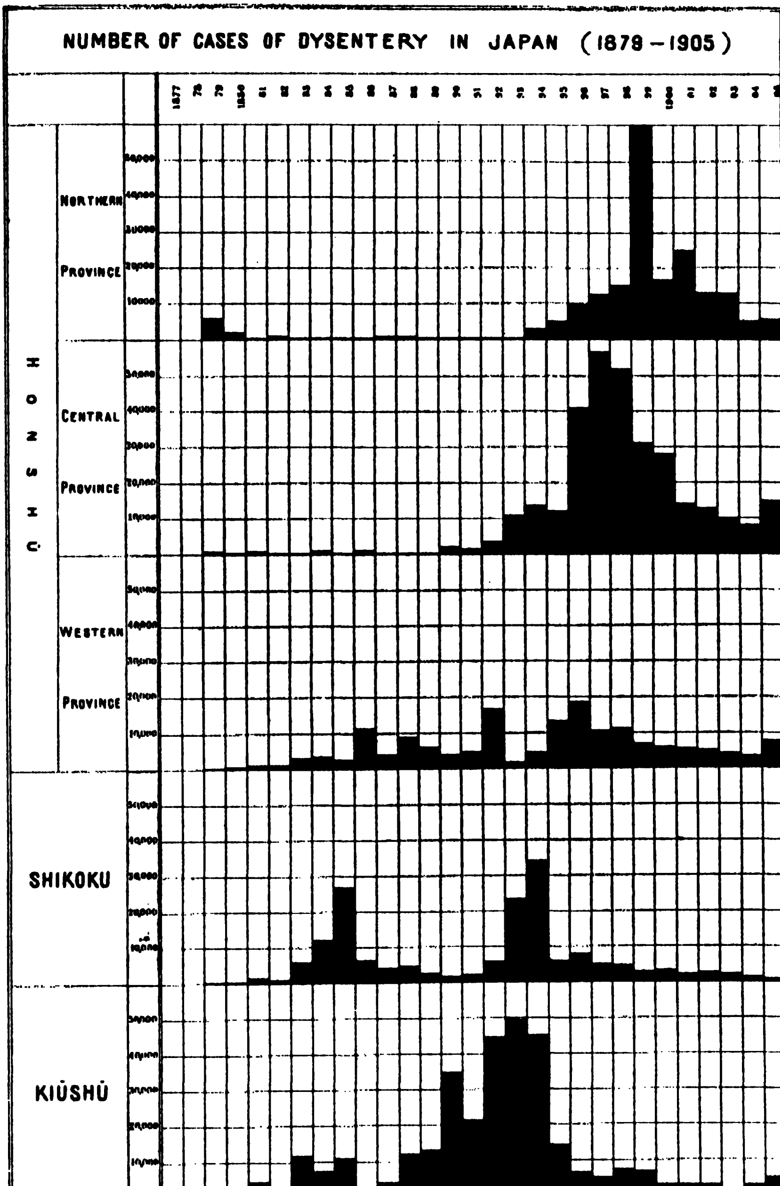

NUMBER OF CASES OF DYSENTERY IN JAPAN (1878-1905)
HONSHU
NORTHERN PROVINCE
CENTRAL PROVINCE
WESTERN PROVINCE
SHIKOKU
KIUSHU

TABLE I.

THE VARIETIES OF THE DYSENTERY BACILLI
(ACCORDING TO DR. OHNO).

	No	Dextrose	Mannite	Maltose	Saccharose	Dextrin	Lactose
Original bacillus	1	+	−	−	−	−	−
	2	+	−	+	−	−	−
	3	+	−	−	+	−	−
	4	+	−	−	−	+	−
	5	+	−	−	−	−	+
	6	+	−	−	+	+	+
Varieties (acid-form)	7	+	+	−	−	−	−
	8	+	+	−	+	−	−
	9	+	+	+	+	−	−
	10	+	+	−	−	+	−
	11	+	+	−	+	+	−
	12	+	+	+	+	+	−
	13	+	+	−	−	−	−
	14	+	+	+	−	−	+
	15	+	+	−	+	+	+
	16	+	+	+	+	+	+

TABLE II.

TYPES OF DYSENTERY BACILLI

		INDOL	DEXTROSE	MANNITE	SACCHAROSE	MALTOSE	DEXTRIN	LACTOSE
TYPE I (ORIGINAL DYSENTERY BACILLUS)		−	+	−	−	−	−	−
TYPE II OR 1	VARIETIES	+−	+	+	−	−	−	−
TYPE III OR 2		+	+	+	+	−	−	−
TYPE IV OR 3		+	+	+	+	+	+	−
TYPE V OR 4		+	+	+−	+	+	+	−

TABLE III.

Dysentery Bacillus	Rabbit Immune-Serum				
	Type I	II	III	IV	V
Agglutination					
Type I	1600	-25	-25	-25	-25
" II	400	6400	3200	800	400
" III	-25	6400	6400	200	800
" IV	100	400	400	6400	800
" V	-25	100	400	1600	3200
Bacteriolysis in Vitro					
Type I	0.0035	0	?	0	0
" II	trace	0.0035	?	0.01	0
" III	?	?	?	?	?
" IV	0	0	?	0.001	0
" V	0	0	?	trace	0.001

TABLE IV.

TYPES OF DYSENTERY BACILLI ENCOUNTERED IN KOBE IN 1905 (ACCORDING TO DR. AMAKO).

	TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TOTAL
JANUARY	0	0	0	1	0	1
FEBRUARY	0	2	0	3	0	5
MARCH	0	0	2	1	0	3
APRIL	0	0	0	2	0	2
MAY	0	3	3	2	0	8
JUNE	5	0	4	3	0	12
JULY	26	43	36	7	0	112
AUGUST	44	79	70	5	3	201
SEPTEMBER	23	53	38	3	6	123
OCTOBER	10	21	13	5	0	49
NOVEMBER	0	3	1	3	0	7
DECEMBER	0	0	2	1	0	3
TOTAL	108	204	169	36	9	526
MORTALITY	13.8%	13.2%	11.8%	8.3%	11.1%	12.3%

TABLE V.

TESTS OF THE POLYVALENT IMMUNE SERA FROM THE HORSE

Immune serum from	Animal experiment	Type I	Type II	Type IV	Type V
Type I	Titer	0.001	0	0.01	0.05
	Bacteriolysis	0.00035	0.001	0	0
	Agglutination	1:1000	1:400	1:1600	1:200
Type II	Titer	0.01	0.001	0	0
	Bacteriolysis	0.0035	0.00035	0	0
	Agglutination	1:800	1:1000	1:800	1:400
Type I and II	Titer	0.001	0.0025	0.01	0.05
	Bacteriolysis	0.001	0.001	0	0
	Agglutination	1:200	1:1000	1:800	1:400
Type I and IV	Titer	0.01	0	0.001	0.0025
	Bacteriolysis	0.001	0	0.0035	0
	Agglutination	1:800	1:200	1:800	1:100
Type I and V	Titer	0.0025	0	0.0025	0.001
	Bacteriolysis	0.001	0	0.001	0
	Agglutination	1:200	1:400	1:400	1:400

TABLE VI.

A CONSIDERATION OF SOME OF BAIL'S RECENT VIEWS IN CONNECTION WITH THE STUDY OF IMMUNITY AND A COMPARISON OF THE VALUE OF PROTECTIVE INOCULATION WITH AGGRESSIN WITH THAT OF VACCINATION IN PLAGUE.[1]

By Richard P. Strong.

(*From the Biological Laboratory, Bureau of Science.*)

One of the more important studies on the subject of immunity appearing during the past year, and one which has attracted a large amount of attention, has been that of Bail,[2] published in several recent numbers of the *Archiv. für Hygiene*. In the introduction to his first paper, after discussing the question of the relationship between bacteriolysis and immunity, he suggests that either the immunity which enters into the animal body in the process of obtaining a bacteriolytic serum represents a false immunity—i. e., not a true resistance against the disease itself, but only one against its cause in the particular form employed—or that in reality it represents a true immunity, the special nature of which, however, arises in something else than bacteriolysis, which is merely an accompanying phenomenon. He further calls attention to the fact that during almost eight years, experiments have been carried on with the expectation of being able to explain the non-infective power of an animal through the processes of bacteriolysis, but he points out that apparently the conclusion should be reached that the germ-killing properties of the body fluids are primarily without value in interpreting the actual processes of immunity. In support of his views attention is called to the fact that the rabbit, whose blood serum usually possesses a powerfully antagonistic action against the anthrax bacillus, is susceptible to the disease anthrax, while the fowl, whose blood shows the opposite properties, is insusceptible. He also alludes to the fact that while immune bodies against this affection could usually be demonstrated in the blood serum of other animals, the proof of a regular relationship

[1] Read March 3, 1906, before the third annual meeting of the Philippine Islands Medical Association.

[2] Oskar Bail: *Archiv. für Hygiene* (1905), **52**, 272; **53**, 275; **53**, 302. Also *Deutsche Medizin. Wchnschr.* (1905), **31**, 1471, 1788.

between the quantity present and the susceptibility of the particular animal in question has failed entirely. Thus, for example, the serum of the sheep often contained very small amounts of immune bodies, that of the rabbit very much larger ones, and that of cattle extraordinarily large quantities of these substances. However, all of these animals have a nearly equal susceptibility for anthrax.

Bail in addition, calls attention to the very significant fact that those experiments, performed with bacteriolytic serum in the test tube, in which in order to approximate the conditions encountered in the animal body the cells of fresh organs were added to the serum, revealed the interesting result that the bactericidal properties of the serum disappeared or were greatly diminished. A somewhat similar phenomenon had also been observed in hæmolysis by Von Dungern,[3] Wilde,[4] Hoke,[5] and others which was explained by these authors as being due to other processes. However, Bail insists that it is of little importance whether this ineffectiveness is conditional upon a failure of immune bodies or of complements, since the chief fact remains that the phenomenon of bacteriolysis is immediately suspended as soon as there exists in the test tube a condition somewhat similar to that encountered in the internal organs. In opposition to Von Dungern and Wilde he claims that there are many reasons why this process of bacteriolysis, which results in the test tube, must fail in the animal body, and he emphasizes the warning that, in every experiment, a careful consideration should be given to the results before the conclusion is reached that the bacteriolysis to be observed *in vitro* will explain the relationship which exists in the animal body. He also calls attention to the fact that in the case of anthrax it was soon shown that neither within the body of the naturally immune fowl nor in that of the artificially immunized rabbit did any process occur which resembled the bacteriolysis observed in the test tube with anthrax baccilli and immune serum, nor did an anthrax protective serum from another animal possess any bactericidal properties against this organism. Therefore, he reasons that the idea of the possession by an animal of a blood and serum with bacteriolytic powers being looked upon as the reason either for its artificial or natural immunity against anthrax must be abandoned. Bail admits that, after a certain time, the death of the inoculated organisms must take place in the body of an immune animal, but he maintains that this process is the result not so much of the action of the body fluids, as of that of certain cells, particularly those of the bone marrow, and that it is not, as is the case where serum in a test tube is used, a quick one in which large numbers of bacteria are killed but on the contrary a relatively slow process accompanied by a

[3] V. Dungern: *München Med. Wchnschr.* (1900), **57,** 677.

[4] Wilde: *Berl. klin. Wchnschr.* (1901), **38,** 878; *Archiv. für Hygiene* (1902), **44,** 1.

[5] Hoke: *Centralbl. für Bakteriol.* Orig. (1903), **34,** 692.

gradual diminution of the organisms. In addition, he concedes that the observations outlined above were formerly considered to be of value only in regard to anthrax, but he argues that since the certainty of bactericidal immunity in anthrax formerly seemed to be generally accepted, that now, from its failure in this disease, doubt was thrown upon the value of bacteriolysis in other infections—for example, in typhoid and cholera. The author refers to the further work of Hoke,[6] who in an extensive series of experiments with different bacteria, demonstrated that when the bacteriolysis was performed in the test tube in such a manner that the conditions approximated the relationship which existed within the organs of an animal, the natural germ-killing power of rabbit serum either was greatly diminished or was entirely eliminated; and further, that after the addition of cells of organs to an entirely fresh serum, not only as in the case of anthrax did the action of the immune bodies fail but that of the complements as well. If, argues Bail, the bacteriolytic action of normal serum against the typhoid bacillus according to these experiments occurs with such difficulty in the rabbit's body, then just as little as in the case of anthrax can the natural bacteriolysis possessed by the blood of this animal against this bacterium be considered as the reason for the natural resistance of the rabbit against typhoid infection; and also, since according to the generally accepted opinion, the development of artificial immunity in typhoid depends only upon this definite tendency toward an increasing bacteriolytic power in the body fluids, then this power must either behave in a manner entirely different from the bacteriolytic one of normal serum, or it also can not be regarded as a reason for the explanation of the immunity. However, it has from the first clearly been shown that no difference in action between the two could be demonstrated, at least in test-tube experiments.

Bail next compares the phenomena of resistance and of immunity and cites experiments in which by the injection of bouillon, of different bacilli, etc., into the abdominal cavity of guinea pigs, insusceptibility against a number of different organisms was obtained. An exudate containing many leucocytes usually results from these injections and although Pfeiffer[7] has pointed out that such inflammatory effusions are rich in amboceptors which bind the bacterial receptors, Bail maintains that in such fluids the most marked, active process causing the destruction of the bacteria is not a bacteriolytic but a phagocytic one and that the bacteriolysis carried on outside of the cells is comparatively insignificant. In support of this statement he describes two experiments in detail. In the first, a guinea pig was given a small dose of cholera immune-serum subcutaneously and at the same time one of living cholera spirilla intraperitoneally. In the second, another animal received exactly the same

[6] Hoke: *Ztschr. für Heilk.* (1904), **25**, 197.

[7] Pfeiffer: *Kongress in Brussels, Pfeifferschen Referates und Schlussfolgerung*, **17**, 25. Ref. Bail, *Archiv. für Hygiene* (1905), **52**, 278.

inoculation as the first, but in addition 5 cubic centimeters of aleuronat was previously injected into the abdominal cavity. The second animal remained alive, and there was marked evidence of extensive phagocytosis in the abdominal cavity that did not occur in the case of the first animal, which died.

Bail assumes the position that this difference in behavior was dependent in the second case not upon an increased killing power of the fluids outside of the cells—i. e., upon bactericidal action—but that the resistance of the animal depended upon the degree of phagocytosis. He then describes experiments to show that, whereas the destruction of the typhoid bacillus under the proper influences may take place very quickly within the abdominal cavity of a guinea pig, within the organs of the same animal an identical result is certainly not observable.

In his further attack upon the significance of bacteriolysis, he points out that sera, such as were produced by Sobernheim in the treatment of animals with anthrax œdema and which showed high protective value, evidenced neither agglutinating nor bactericidal properties: whereas rabbits and large guinea pigs, inoculated subcutaneously or intraperitoneally for a long period with anthrax cultures grown at 42° C. or carefully killed, furnished sera which had a distinct if not very powerful agglutinating action (1–500), but, although *in vitro* their value in immune bodies was about three times this strength, they revealed no protective power for rabbits, even when the serum was injected at the same time with the bacteria. Moreover, the animal which furnished the best serum possessed no true immunity, since it succumbed within three days after a subcutaneous inoculation of less than 1,000 anthrax bacilli. Bail claims a somewhat similar relationship in typhoid fever and cholera, though he admits that there are certain differences with these diseases, owing to the fact that the cholera spirillum and the typhoid bacillus belong rather to the group of half, or facultative invasive, parasites for animals, or indeed, perhaps stand nearer to the saprophytes in contradistinction to the anthrax bacillus, which may be considered as a true invasive parasitic organism. He argues that when the above facts are all taken into consideration it is easy to understand why agglutinating and bacteriolytic sera may be obtained by the inoculation of an entirely avirulent cholera culture; since in this instance also we obtain immunity against the bacillus but not against the disease itself.

After insisting further upon the fallacies of the consideration of bacteriolysis in its relation to true immunity, many of the details of which can not be entered into here, Bail takes up the discussion of the "aggressins." As is well known, Kruse[8] originally particularly advocated the idea that, in their various relations with living tissues, pathogenic bacteria

[8] Kruse: *Beitr. zur path. Anat. u. z. allg. Path.* (1893), **12**, 333. See also Bail: *Centralbl. für Bakteriol.* Orig. (1904), **36**, 266, 397.

form chemical substances in their protoplasm which so act upon the cells and fluids of the animal for which the organism is pathogenic that they overcome its natural resistance against the infection. This author at first designated these substances as lysines, but, owing to the fact that this term came to be employed in a different sense, he together with Bail more recently proposed for them the name of "aggressins."

Bail also points out that a bacillus must injure and overcome the animal's natural protective power in order to survive in the body of an animal and produce its particular disease. This it is able to do by means of its aggressive properties, which depend upon a certain substance which may be designated as "aggressin." These aggressins he believes are only to be formed by the bacteria in the animal body and they are particularly to be encountered in pathological fluids such as œdemas and exudates which result from the inoculation of the different micro-organisms. Thus, anthrax aggressin is contained in the œdematous fluid resulting from the subcutaneous injection into animals of the anthrax bacillus, whereas in the exudate which forms in the abdominal or pleural cavity of the rabbit or guinea pig, following the inoculation of the typhoid bacillus or cholera spirillum, typhoid or cholera aggressin is met with. He maintains that when these different exudates were carefully centrifugalized and thus freed from all the animal cells and most of the bacteria, and were then sterilized, in each instance a clear, yellow fluid resulted which contained the particular aggressin. For these substances he emphasizes the following distinctions:

First. Non-lethal amounts of cholera and typhoid bacteria, when injected into the animal simultaneously with the corresponding aggressin, cause the death of the animal; that is, the aggressin stimulates the bacteria to the production of toxic substances in such quantities that the animal succumbs.

Second. Fatal doses of these bacteria, which, however, give rise only to comparatively mild or subacute infections, produce with the aid of aggressin very severe and acutely fatal ones; hence the aggressin must aid the bacteria favorably in their struggle against the animal during the course of the infection.

Third. By injections of aggressin into the abdominal cavity of animals, the protective action of a bactericidal serum may be suspended.

Fourth. By the injection of aggressin an immunity is obtained which differs widely from bactericidal immunity.

I believe Bail has by his extensive experiments undoubtedly demonstrated that results somewhat similar to those outlined in these conclusions occur for several infections, although it would appear that in typhoid fever and cholera his so-called aggressin immunity depends chiefly upon bacteriolysis. However, as I shall point out further on, the results of all of his work with these substances are apparently in great part dependent upon the action of and explained according to entirely different processes

from those he has outlined. Noteworthy among other specific qualities mentioned for the aggressins is the fact that heating for one-half hour at 55° to 60° destroys their action, while sterilization with chloroform, toluol, and dilute solutions of carbolic acid weakens it. Bail found that injections of aggressin alone were not particularly poisonous and never acutely caused death in animals. In case a fatal dose had been inoculated, there was always a prolonged illness with marked loss of weight. Not all of the exudates he produced contain aggressin an it was found to vary considerably in quantity in the different ones, but was usually likely to be present in largest amounts in the effusions which were rich in cells.

In his conclusions in regard to the aggressins he emphasizes the fact that their action can not be explained on the assumption of their being anti-complements or anti-immune bodies, soluble bacterial substances, free receptors, etc., but they must be considered as new substances which have heretofore been unrecognized and which are formed by the bacteria in the living organism during the course of the infection.

The great practical importance of Bail's observations, apart from their theoretical interest, can immediately be seen. Indeed, what he has maintained is that our ideas in regard to immunization with a number of different bacteria, and our method of obtaining immune sera, are erroneous; that in the production of such sera we have treated the animals merely with the bacteria or with bacterial products obtained in artificial and inanimate media, and not with the weapons by means of which the bacteria carry on the combat in the living body, namely, by means of aggressins. In this manner he claims sera to have been obtained which act upon the bacteria but not upon the aggressin, and he even goes further and states that a true immunity against the disease—that is, a really active serum—can only be obtained from the injection of the living organism under the influence of aggressin. In this manner, not an anti-serum against the bacillus but one against the aggressin, hence an anti-aggressin serum, is produced. Therefore, for the production of the true immunity he insists that the animal must be inoculated with aggressin—i. e., with the body fluids of an animal which has succumbed to the particular infection.

As has been mentioned, the work of Bail soon attracted the attention of other observers, and both Wassermann and Pfeiffer published articles relating to his studies. Pfeiffer and Friedberger, in their recent work entitled "Weitere Untersuchungen Ueber die Antagonistische Wirkung Normaler Sera,"[9] have particularly considered the statement of Bail that, by intraperitoneal injections of an animal exudate containing aggressin into a guinea pig, the action of a bactericidal immune serum introduced at the same time could be entirely suspended. That such a suspension of the bacteriolytic process of an immune serum does take place within

[9] Pfeiffer und Friedberger: *Deutsche Medizin. Wchnschr.* (1905), **31**, 1145.

the abdominal cavity of an animal, when the bacteria which were tested were previously treated with normal serum of another animal. Pfeiffer and Friedberger had already previously shown.[10] In their most recent article they emphasize that this phenomenon can not be explained upon the aggressin hypothesis of Bail. They also show that it can not be elucidated upon the assumption that, when the normal serum is added to the bacteria, soluble substances, the free receptors of the organism, are taken up by the serum; and moreover that its action does not coincide with the theory of Sachs of complement deviation, which was suggested for the explanation of what at first appeared to be a similar process observed in hæmolysis.

Pfeiffer and Freidberger on the contrary believe that this antagonistic action represents the fundamental properties of a normal serum, and the subject therefore is one of very great significance in the processes of immunity. While it must be added that all observers do not accept their explanation for this phenomenon, notably among them Gay,[11] who believes that the explanation lies in the fact that specific serum-precipitates which are able to fix alexine are present, nevertheless it seems entirely clear that the process can not be satisfactorily explained according to the aggressin theory of Bail. Wassermann and Citron[12] in their paper have considered the question which is apparently of most practical importance in its relation to Bail's studies, namely, whether in the infected organism, in the conflict between the invading bacteria and the cells of the host, a substance, aggressin, actually is formed, and whether this in reality is a new substance and one which, as yet, we have not been able to demonstrate in our investigations of the living bacteria outside of the body.

As Wassermann has remarked, if this is the case then the further conclusions of Bail must be acknowledged, namely, that by means of aggressin an anti-aggressin serum can be obtained which will possess different qualities from those sera previously obtained by other methods and which, perhaps, may act against the infection in a more favorable manner. Wassermann and Citron first performed experiments relating to this subject in exactly the same manner as Bail had recommended and found that they could confirm his work. They next investigated the question as to whether these substances, termed aggressins, were to be found only in the living organism as a result of the infection. Their experiments were performed with typhoid, hog-cholera, and swine-plague bacilli. Sterile exudates produced in rabbits by the injection of aleuronat were collected in glass vessels in an aseptic manner and their sterility proven. The living bacteria were then added to the exudates and were placed in an incubator at 37° for twenty-four hours. The mixtures were next centrifugalized and the clear fluid drawn off from above and sterilized.

[10] Pfeiffer und Friedberger: *Deutsche Medizin. Wchnschr.* (1905), **31**, 6.
[11] Gay: *Centralbl. für Bakteriol.* Orig. (1905), **39**, 603.
[12] Wassermann und Citron: *Deutsche Medizin. Wchnschr.* (1905), **31**, 1101.

These exudates, after this treatment, showed exactly the same aggressin action as those which were produced by the occurrence of the infection in the living animal. Similar experiments were now performed with rabbits' normal *blood* serum to which the bacteria were added (*in vitro*), the mixtures being further treated in the same way. These sera also showed properties similar to those of the aggressins. Finally, the bacteria were merely suspended in distilled water, thoroughly shaken, sterilized, and separated by centrifugalization from the fluid; the watery extracts which were thus obtained and which contained the soluble substances of the bacteria also possessed exactly the same action as did Bail's aggressin exudates. Therefore, Wassermann and Citron conclude that the so-called aggressins of Bail have nothing to do with the living animal organism and that they are not new bodies but merely dissolved bacterial substances, with the immunizing properties of which we have long been familiar.

Shortly after the appearance of his first paper, Bail and his pupils and associates published further articles demonstrating that high and undoubted immunity could be obtained in animals by the inoculation of these so-called aggressin exudates. Bail demonstrated such results for typhoid, cholera, and anthrax infection, while Kikuchi [13] showed that by the use of dysentery aggressin guinea pigs could be immunized against infection with dysentery bacilli and rabbits against the action of the dysentery toxin. Still more important were the results obtained by Weil [14] in the immunization of animals against chicken cholera with aggressin exudates. The difficulties in obtaining active immunity against hæmorrhagic septicæmia have for a long time been well recognized. Inoculations with attenuated, living cultures have proved to be very unsatisfactory and Voges,[15] using killed cultures, obtained unfavorable results. However, Kitt,[16] who employed the œdematous fluids from animals dead of the affection, had better success. The production of a protective immune serum for this disease has also been difficult; indeed, several observers have maintained that hitherto a method of obtaining such a satisfactory serum has not been known. However, with the exudates containing aggressin, collected from the pleural cavity of animals infected with chicken cholera bacilli, Weil was able to immunize other animals and to obtain a protective immune serum of high potency. He points out that the action of this serum is not bacteriolytic but that it possesses anti-aggressive properties. As intimated, Weil's experiments

[13] Kikuchi: *Archiv für Hygiene* (1905), **52**, 378. *Ibid.* (1905), **54**, 297. See also the articles of Von Hoke on "Aggressive wirkung von Pneumokokken und Staphylokokken Exsudat," *Wiener Klin. Wchnschr.* (1905), **18**, 348, and *Ztschr. für Hygiene* (1905), **50**, 541, and of Salus "das Aggressin des Colibacterium," *Wiener Klin. Wchnschr.* (1905), **18**, 660.

[14] Weil: *Archiv. für Hygiene* (1905), **52**, 412. *Ibid.* (1905), **54**, 149.

[15] Voges: *Ztschr. für Hygiene* (1896), **23**, 149.

[16] Kitt, Kolle, und Wassermann: *Handbuch d. pathogenen Mikroorganismen* (1904), **4**, 969.

have also demonstrated that, by the injection of the aggressin exudates into animals, a higher immunity against chicken cholera resulted than had been obtained by any other method.

However, hardly three months ago, Citron,[17] working in Wassermann's laboratory, published experiments which showed that with sterilized aqueous extracts of the hog-cholera and swine-plague bacillus, he was also able to obtain a similar immunity in animals and to produce an immune serum which was of equal value with and which acted in a manner exactly similar to an anti-aggressin serum. He points out that the aggressin immunity is therefore not an immunity *sui generis*. Finally, in the last paper to appear from Hueppe's Institut (in which laboratory all the work of Bail and his associates has been carried on), the announcement is made by Hueppe and Kikuchi[18] that successful results have been obtained in the immunization of guinea pigs against plague infection by repeated inoculations with exudates containing plague aggressin. The experiments which are related briefly are very few in number, but the authors mention that in this paper it is their intention merely to call attention to the priority of the use of this method of immunization of animals against plague.

Up to the present time we were aware of no other reasonably sure method of immunizing guinea pigs against plague infection except by that one in which the living, attenuated plague bacillus was inoculated. Upon this subject I have recently reported in detail in the PHILIPPINE JOURNAL OF SCIENCE.[19] From numerous experiments on animals I had found that the living, attenuated plague bacillus caused higher immunity than the dead cultures of this organism in any form. However, while the method of *vaccination* was by far the most favorable one for practicable immunization, it could not perhaps, be said to be absolutely satisfactory from an experimental standpoint, because the *single* inoculation of a large dose of an avirulent plague culture, of such attenuation that it is never capable of causing the death of an animal even in very much larger amounts, does not invariably protect the animal against subsequent plague infection. Thus, with monkeys, from 60 to 80 per cent of the different series of those vaccinated with such a culture possessed high immunity and survived a subsequent inoculation of a multiple lethal dose of the virulent pest bacillus, but the remaining 20 to 40 per cent were not thoroughly immunized by the single vaccination and succumbed to the subsequent injection of the virulent organism. By using more virulent cultures for vaccination a higher percentage of animals was protected. However, in order thoroughly to immunize a greater proportion of the monkeys, it was necessary to use cultures of such virulence that

[17] Citron: *Centralbl. für Bakteriol.* Orig. (1905), **40**, 610.
[18] Hueppe und Kikuchi: *Centralbl. für Bakteriol.* Orig. (1905), **39**, 610.
[19] Strong: *Philippine Journal of Science*, (1906), **1**, 181.

some of the animals succumbed from the result of the vaccination itself—i. e., they died of a true plague infection, hence the limit of safety had been passed. Naturally, only cultures which were invariably non-fatal for animals could be employed or recommended for use in human beings, and with these cultures it has been possible to render immune, by a single inoculation and a moderate dose, only from 60 to 80 per cent of the experimental animals.

Therefore, when the announcement was made that, by immunization with repeated doses of aggressin, guinea pigs could also be protected against plague infection, the importance of this claim was readily appreciated, and I determined to convince myself of the value of this method and at the same time to discover if guinea pigs, perhaps the most susceptible of all animals to plague, could not be immunized with certain bacterial extracts of the plague bacillus, particularly since the method of immunization with animal exudates as it is recommended by Bail can not be considered practicable for the inoculation of large numbers of people. In this connection it may be mentioned that by experimental work I had already found that an extract of the plague bacillus obtained in a manner similar to that already employed in this laboratory in the manufacture of our cholera prophylactic [20] from the cholera spirillum was not sufficiently potent, since not over 40 per cent of the animals inoculated with large doses thereof proved to be fully immune. I therefore determined to modify this method and to employ such means as would injure the chemical substances of the plague bacillus as little as possible during the process of extraction. For this purpose a procedure similar to that suggested and used by Wassermann [21] for the purpose of obtaining immunizing substances from typhoid and hog-cholera bacilli, and employed in a modified manner by Brieger, Bassenge and Meyer,[22] was chosen.

Cultures of a strain of the plague bacillus of the highest obtainable virulence were grown upon the surface of large test tubes of agar during forty-eight hours at 30° C. The growth was then suspended in distilled water, 1 cubic centimeter of water being employed for each 30 milligrams of bacteria (a very concentrated suspension). The resulting mixture was then placed in a sterilized bottle and fastened upon an electrical shaking machine and thoroughly shaken for five days. At the end of this time it was removed and heated at 44° to 45° C. for one

[20] Strong: *American Medicine* (1903), **6**, 272; *Publications Biological Laboratory, Bureau of Government Laboratories* (1904), **16**, 1; *Journal of Infectious Diseases* (1905), **2**, 107.

[21] Wassermann und Citron: *Deutsche Medizin. Wchnschr.* (1905), **31**, 1102.

[22] Brieger: Sonderabdruck aus den *Verhandlungen des deutschen Kolonialkongresses* (1905), Sektion **2**, 182; also Brieger und Meyer, *Deutsche Medizin. Wchnschr.* (1904), **30**, 980; Bassenge and Meyer, *Centralb. für Bakteriol.* Abt. 1 (1904), **36**, 332.

hour, for the purpose of killing the remaining living bacteria; 0.5 per cent of carbolic acid was then added in order to complete the sterilization. After standing twenty-four hours the fluid was centrifugalized for five hours at a velocity of 4,000 revolutions a minute. The clear fluid above was then pipetted from the bacterial sediment.

The liquid obtained by this method is perfectly clear and amber colored, and, if the process has been carried on with sufficient care, it is sterile. Intraperitoneal inoculations into small guinea pigs of as large a dose as 5 cubic centimeters has in these animals in several instances produced convulsions which lasted for an hour or more but from which the animals have always recovered. It seems very likely that these symptoms owed their origin rather to the small yet appreciable amount of carbolic acid present, rather than to the extracted substances of the bacilli. A number of experiments have been performed in which *single* injections of this fluid in doses of from 2 to 5 cubic centimeters in guinea pigs and from 1 to 5 cubic centimeters in monkeys have been made. Since the development of pest immunity takes place slowly, the animals were not tested with the virulent pest organism until fifteen days after the injection of the fluid. In my last series of inoculations with such an extract, comprising fifty-three animals, none of the guinea pigs [23] and but 25 per cent of the monkeys, on subsequent inoculation with a virulent plague bacillus, proved to be immune. Therefore, the results in immunization with this extract of the plague bacillus in this series of experiments at least are not nearly as good as those obtained with the living organisms of sufficient attenuation to be used for human inoculation. Hence, immunization with the attenuated living plague bacillus must be acknowledged as being superior to that in which these extracts are employed, and therefore vaccination must still be considered

[23] The method employed in testing the immunity of the guinea pigs in these experiments was a severe one. The shaved abdomen of the animal was scarified with a sharp knife, three parallel incisions being made through the dermis. The abdomen in this region was then thoroughly massaged with a portion of a spleen of a guinea pig which had died a few hours previously of plague infection produced with a plague organism which had passed successively (without growth on artificial media) through over 150 animals. Portions of the *same* spleen which were known to contain very large numbers of plague bacilli were used for all the inoculations. The abdomen was scarified and the test thus made severe in order to demonstrate whether this method of immunization in which the extracted substances of the bacillus were employed was superior to that in which the living attenuated organism was used. However, these experiments are not to be considered as final in relation to the value of immunization with the free receptors of the plague bacillus as a further study of this subject with certain modifications of method, is at present being pursued. Moreover, having failed to immunize guinea pigs with single injections of this extract, I shall naturally now endeavor to demonstrate whether it is possible to immunize these animals with repeated doses, both of it and of the animal exudates such as Hueppe and Kikuchi employed.

the most satisfactory method for use in human protection against plague. However, my experiments have shown that with an extract of the plague bacillus I have been able to produce in monkeys a certain degree of immunity against plague infection, similar to that brought about by Hueppe and Kikuchi with peritoneal exudates from animals dead of plague, but since the extract I have obtained, while it exerts an action similar to that of the so-called aggressin exudates, is prepared by a different method, namely, from the bacteria which have been cultivated outside of the animal body and which have not been acted upon by an animal exudate, I shall propose to call it merely plague bacillus extract. In regard to the use of the term "anti-aggressin" for those sera obtained during the process of immunization against such diseases as anthrax, rinderpest, plague, etc., and in which diseases it has long been recognized that there is no true bacteriolytic immunity, it would appear that the previously proposed term of anti-infectious sera would answer as well if not better. The aggressins in spite of the work of Bail and his associates, must still be regarded as hypothetical substances.

A STUDY OF THE BLOOD IN DENGUE FEVER WITH PARTICULAR REFERENCE TO THE DIFFERENTIAL COUNT OF THE LEUCOCYTES IN THE DIAGNOSIS OF THE DISEASE.

By E. R. Stitt.[1]

(*From the Laboratory of the United States Naval Hospital, Canacao, P. I.*)

Dengue resembles a composite photograph in that it is by adjusting and subordinating the symptomatology that one arrives at a diagnosis of the disease—the moment one symptom stands out preëminently, there is a strong probability of mistaking dengue for some other affection. In proof of this statement may be instanced a few cases which have been observed during the past six months and in which at first another disease had been diagnosed.

1. A case presenting unusually severe pains about the regions of the knees and wrists. This was diagnosed as acute articular rheumatism. A careful examination failed to show any swelling and the pain was more associated with the structures adjacent to it than with the joint itself. A rapid survey of the other, but less pronounced, symptoms, together with the blood examination, showed the case to be one of dengue.

2. A case where there was a gradual, step-like rise of temperature with an unusually heavily coated tongue and a marked right iliac tenderness. It was only when a most characteristic rash appeared and the temperature fell to normal by crisis that a correct diagnosis was made. It may be stated that negative agglutination caused this case to be very carefully studied, and it was in this connection that the blood changes, to which I shall presently refer, were first observed.

3. A man admitted to the hospital with a most profuse general rash, the case having been diagnosed as measles. From the standpoint of the rash it certainly resembled measles, but a marked *leucopænia* with a rather characteristic differential count, together with the presence of less prominent symptoms, showed the case to be dengue.

4. Two cases in which there was an almost entire absence of eruption, but a history of previous malarial attacks, resembled æstivo-autumnal fever, but the negative evidence as to parasites and the striking difference as to blood findings, made the diagnosis easy.

In fresh dengue blood I have observed most suggestive vacuolation. There seems to be more of what might be termed a protoplasmic milkiness

[1] Read before the third annual meeting of the Philippine Islands Medical Association at Manila, March 2, 1906, by E. R. Stitt, M. D., surgeon and lieutenant-commander, United States Navy.

than is seen in vacuolation as it is ordinarily observed; furthermore, on several occasions I have been sure that I have seen movement, both amœboid and locomotor. Absence of staining reactions and constant watching have made me believe that I was simply observing vacuolar distortion from contraction of the red-cell stroma. I feel sure that an observer who has not repeatedly examined and carefully studied the unstained malarial parasite would in all probability mistake these vacuolations for *Plasmodium malariæ*.

While convinced of the great value of a differential count in the diagnosis of dengue yet, from practically negative findings in two undoubted cases of the disease, I should assign to it simply the place of a striking symptom and would not consider it to be a pathognomonic sign. For this reason it would seem advisable briefly to consider those symptoms which, in my opinion, are the most characteristic.

A valuable analysis of the symptomatology of the disease is to be found in the "Report of the Dengue Epidemic in Brisbane in 1905," [2] and in presenting my views as to the value of the various symptoms, I think I could not do better than briefly to discuss certain paragraphs in this report. Taking the symptoms in detail we have:

1. *Onset.*—This is stated to be sudden. This has been our experience, however, without any case of abrupt onset. There was always a period of several hours separating perfect health from manifestations of the disease. There was nothing approaching the case described by Martialis where one member of a family, who had up to that time escaped the disease, was seized in the midst of a pantomime in which he was mimicking the sufferings of the others.

2. *Temperature.*—This is stated to have ranged between 39° and 39.5° C. and most rarely reaching 40° or 40.6° C., it generally persisted as an initial rise during two or three days, and after a remission which, as a rule, lasted only for a few hours, it was followed by a terminal one continuing for one or two days and falling by crisis.

This has been exactly our experience with the fever, except that at times no remission was observed. Again, the fall by crisis almost always seemed to coincide with the maximum manifestations of the eruptions.

3. *Pains.*—In the report it is noted that post-orbital or frontal headaches were almost always present; that the eyeballs were generally tender to pressure and that lumbar pain was almost as common as the headaches. In addition, many patients complained of pains in the calves of the legs and in the regions adjacent to the joints but never in the joints. Our experience has been that, if we except the pains in the head and eyeballs, there has not as a rule been marked suffering, the patient experiencing more malaise than actual pain. Many of the cases have complained of a feeling as if sand were in the eyes.

[2] *Journ. Trop. Med.* (1905), 8, 355.

4. *Nervous symptoms.*—In addition to the headaches, reference is made to other nervous manifestations such as insomnia and, particularly, depression during convalescence. This depression in our patients when we were assuring them that they were well of the disease, impressed us as a most striking symptom. In no disease, other than influenza, have I seen it so marked.

5. *The eruption.*—The initial eruption usually is reported to have been simply a blotchy flushing of the face and neck which disappeared rapidly. The terminal rash was present, or rather observed, in only about 50 per cent of the cases. It was stated to have been more like the rotheln or measles rash than scarlatiniform in character. This report refers to its predilection for the extensor surfaces of the elbows and knees and also to a carmine-pink suffusion of the palms and soles, which was regarded as being extremely characteristic.

In our cases the most striking feature of the eruption has appeared to be its early and marked appearance about the wrists, on which it was more abundant on the dorsal than on the flexor surfaces. I commonly describe it as the bracelet rash, having a wide, dorsal outline and a narrower ring of eruption underneath. We have frequently noted the rash on the palms of the hands, but it has seemed to resemble the erythematous syphilide of the palms more than what should be termed a suffusion. In only one of the cases was there an entire absence of secondary eruption; the patient being a Chinaman in whom the other symptoms and the differential count were quite characteristic. Stedman states that he has invariably failed to find the rash in dengue cases among the Chinese.

The symptoms described in connection with changes in the tongue, lymphatic glands, alimentary canal, renal and respiratory systems were so varied that little weight could be attached to them.

It is stated that early in the epidemic discussed in the above-mentioned report many of the cases were diagnosed as influenza and that in the absence of an exanthem in a patient presenting the symptoms of fever, muscular pains and great depression, the best means of diagnosing dengue was found to be in the almost entire absence of respiratory symptoms. It has certainly appeared to me in many respects greatly to resemble influenza.

Shortly after commencing my investigations as to variations in the percentages of the various leucocytes, I was greatly interested in reading a paragraph of an article by Surgeon D. N. Carpenter of the United States Navy [4] in which the author stated that he had found a most characteristic diminution of the polymorphonuclears and a corresponding increase in the small lymphocytes in the blood of dengue patients. This at once attracted my attention, inasmuch as up to that time I had almost

[4] Carpenter, D. N.: *Journ. Am. Med. Asso.* (1905), **45**, **982**.

invariably noted a most marked increase of the large mononuclear leucocytes and transitionals, and almost an entire absence of small lymphocytes. The counts in five cases which had been very carefully studied averaged as follows:

	Per cent.
Polymorphonuclears	42
Large mononuclears and transitionals	40
Large lymphocytes	8
Small lymphocytes	8
Eosinophiles and mast cells	2

In all these cases the counts were made after the appearance of the rash, the patient having been sent to the hospital about the time of the terminal rash. Subsequently, a patient who had been admitted with dysentery developed a temperature of 39.5° C. on the second day after his admission. Suspecting dengue, a blood count was immediately made and recorded as follows:

	Per cent.
Polymorphonuclears	32
Large lymphocytes	25
Small lymphocytes	37
Large mononuclears and transitionals	6

After the appearance of the rash his differential count corresponded closely with the ones previously given.

As opportunity occurred to examine several cases at the commencement of the disease, we were surprised to find in one, during the first day of illness, a count of 50 per cent of small lymphocytes, with very few large lymphocytes and scarcely any large mononuclears. Repeated examinations were made daily in this case and the leucocytes were found to vary markedly from day to day, so that by the fourth one there were only about 8 per cent of small lymphocytes, the latter having been replaced by the large mononuclears and large lymphocytes.

When I refer to a small lymphocyte I mean the leucocyte which constitutes the characteristic element of lymphatic leukæmia and which is largely made up of nucleus, with only a narrow or crescentic fringe of cytoplasm. As a result of my limited experience I believe it to be very probable that, should differential counts be made in a large series of cases, it would develop that the diminution in percentage of polymorphonuclears is largely replaced, during the first day, by small lymphocytes, subsequently, the large lymphocytes would replace the small ones and on about the fifth day, or at the time of the appearance of the terminal rash, it would be noted that large mononuclears and transitionals had taken the place of the lymphocytes. It is this almost kaleidoscopic change in differential count which is so characteristic of dengue and I do not know of any other disease in any degree presenting such a striking variation.

It can readily be seen that the differential leucocyte count would be of very great value in the diseases which might specially be confounded

with dengue. In typhoid it is the rule to have a more or less constant increase in the percentage of the small lymphocytes, while in malaria we are all familiar with the fact that there is a large proportion of large mononuclears. In dengue we have at different periods the characteristics of these two diseases.

The blood has invariably shown a leucopenia, the counts varying from 1,450 to 5,280 per cubic millimeter. This fact, together with the differential count, would easily differentiate scarlet fever and measles.

From the beginning it was my aim in studying the blood of dengue to discover the bodies described by Graham of Beirut.

It will be remembered that this author claimed to have discovered a non-pigmented protozoön in the blood of patients suffering from dengue. He stated that it was constantly changing its position in the blood cell and that it increased from day to day so that by the fifth or sixth one it would be more than one-half the size of the red cell, and diagrammatically he represented it as at times projecting from the cell like a pseudopod. He stated that he was unable to stain these bodies. He also claimed that he found oval and spherical spores in the stomach and salivary glands of culex, these spores appearing forty-eight hours after the mosquito became infected. I have repeatedly and for extended periods searched blood specimens without finding anything which might be termed peculiar. As previously stated, I was struck with the remarkable vacuolations observed in fresh specimens.

My method of work was from time to time to examine fresh specimens, carefully ringed with vaseline, until crenation was so general as to be confusing. Any peculiarity of the red or white cells was noted, and specimens of the blood which were stained in three ways were carefully examined to verify such supposed findings. Films were stained with Wright's blood stain, hæmatoxylin, and eosin, and with thionin. In not a single instance could I find anything which would suggest a protozoön. Occasionally, I also employed prolonged staining with Wright's stain according to the method suggested by Castellani as a substitute for Giemsa's stain as used for *Spirochaeta pallida*.

While I did not have an opportunity to verify Graham's findings in regard to the mosquito, yet there are certain observations which would tend to strengthen his claim that the disease is transmitted by a species of culex. Every case of dengue sent to the Naval Hospital at Canacao has been treated in the wards, yet not once has there been an instance of infection among the large number of non-immunes who were present in the hospital. When the hospital was located at Cavite, it was commonly noted that practically every case admitted became infected with dengue while under treatment for the original complaint. During the present small epidemic in Cavite it has been observed that newcomers, sleeping in Cavite, were frequently infected. Since the admission of our first case in October, 1905, there has been a noticeable freedom from the mosquito pest, so much so that recently, even after carefully searching

the wards, it has been impossible to secure a single one of the insects for examination.

A very striking instance of freedom from attack while on board ship (where mosquitoes are generally rare), and infection when detailed for shore duty for only five days at Cavite, is given by Medical Director Persons, United States Navy, in the case of a squad of marines from the U. S. S. *Baltimore*. Twenty out of twenty-four marines, who had been ashore, contracted the disease after returning to the ship, while there was a total absence of infection among those who had remained aboard.[5]

As to the possibility of a micrococcus being the cause of dengue, as claimed by two investigators in 1885 and 1897, I am unable to give any evidence. However, this matter was investigated and reported upon negatively by the Government Laboratories of the Philippine Islands in 1902.[6] Of one thing I am convinced and that is that the disease is not infectious in the same sense as is influenza.

The claims of Eberle[7] that he found a "dengue plasmœbic organism" which was more minute and more active than the malarial parasite, I do not consider well founded. He states that the motion of the parasite is so swift, it appearing and disappearing so rapidly that, when shown to a microscopist, it is not apt to be seen. He describes and pictures in his diagrams sporulating bodies. The fact that he claims most satisfactory results in staining these bodies with weak eosin and methylene blue would indicate that in stained preparations their discovery should be one of little difficulty. The statement that he found phagocytes literally swarming with the minute organism of dengue (which was one-fifth the size of a red cell), and furthermore, an examination of this appearance in his diagrams, leads me to question whether he might not have been observing an aggregation of blood platelets. In many of my films stained by Wright's method I have seen masses of blood platelets which presented an appearance very similar to that pictured in Eberle's diagram. In conclusion I should consider the most characteristic blood findings of dengue to be the following:

1. Absence of a demonstrable protozoön.
2. Leucopænia.
3. Diminution of polymorphonuclears.
4. A striking variation in the percentage of other leucocytes at varying periods of the disease. At first a large increase in the small lymphocytes is observed, then the appearance of a greater proportion of large lymphocytes, and in the final stages (at the time of the terminal rash and during convalescence) a most striking increase in the mononuclears.

[5] Persons, R. C.: *Journ. Assoc. Military Surgeons* (1905), **17**, 324.

[6] Report of the Director of the Biological Laboratory from the *Annual Report of the Superintendent of Government Laboratories* (1902), 575.

[7] Eberle, H. A.: *N. Y. Med. Journ.* (1904), **80**, 1207.

CURVES SHOWING LEUCOCYTIC VARIATIONS IN DENGUE.

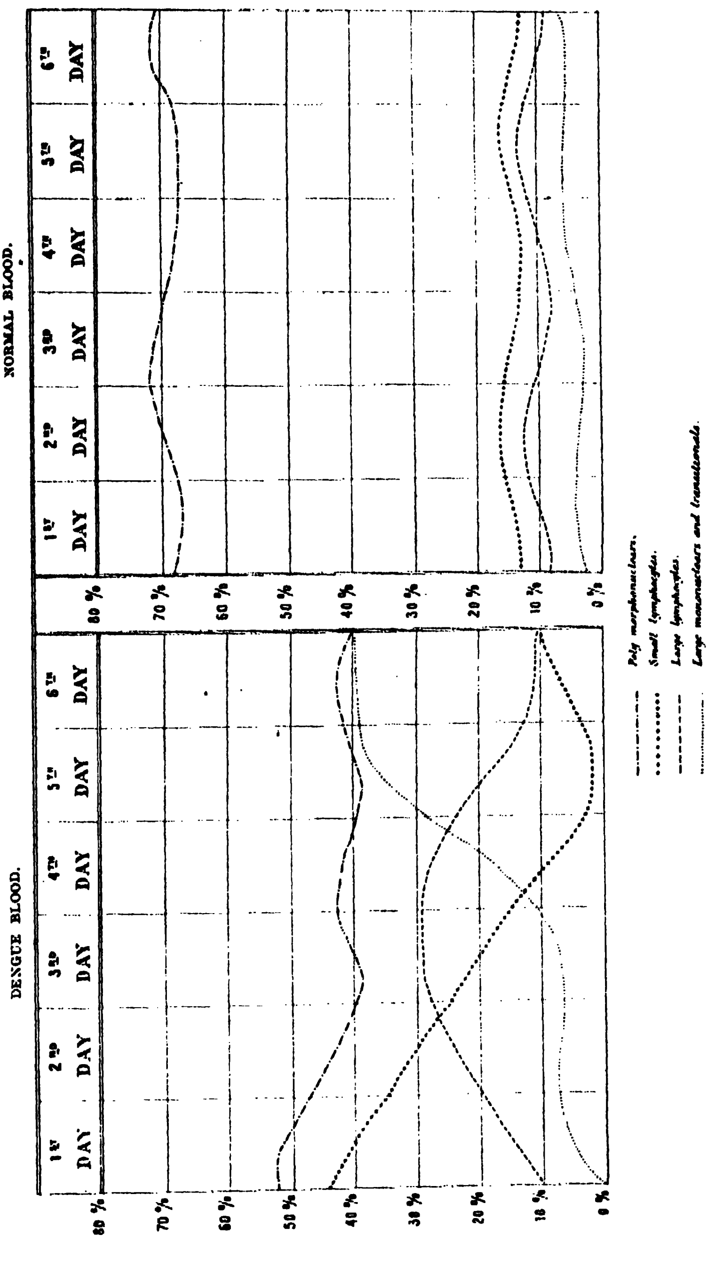


PLATE I.

CURVES SHOWING LEUCOCYTIC VARIATIONS IN DENGUE.

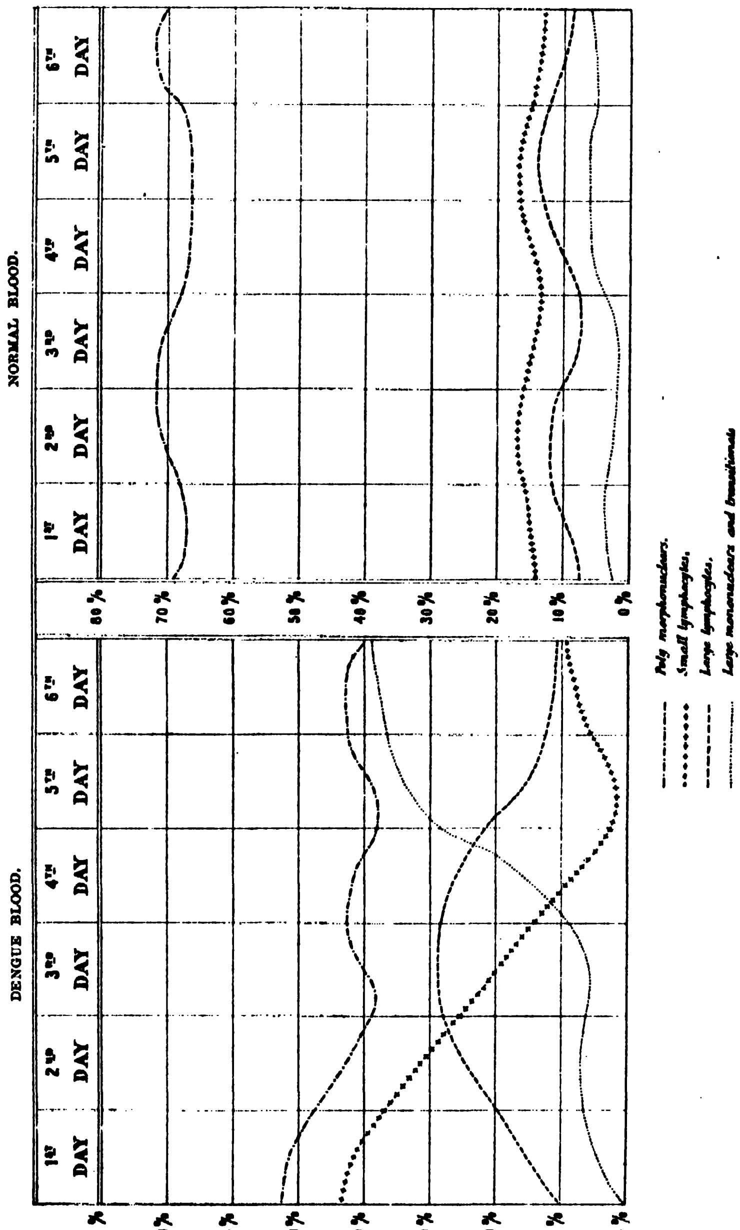


PLATE II.

CURVES SHOWING LEUCOCYTIC VARIATIONS IN DENGUE.

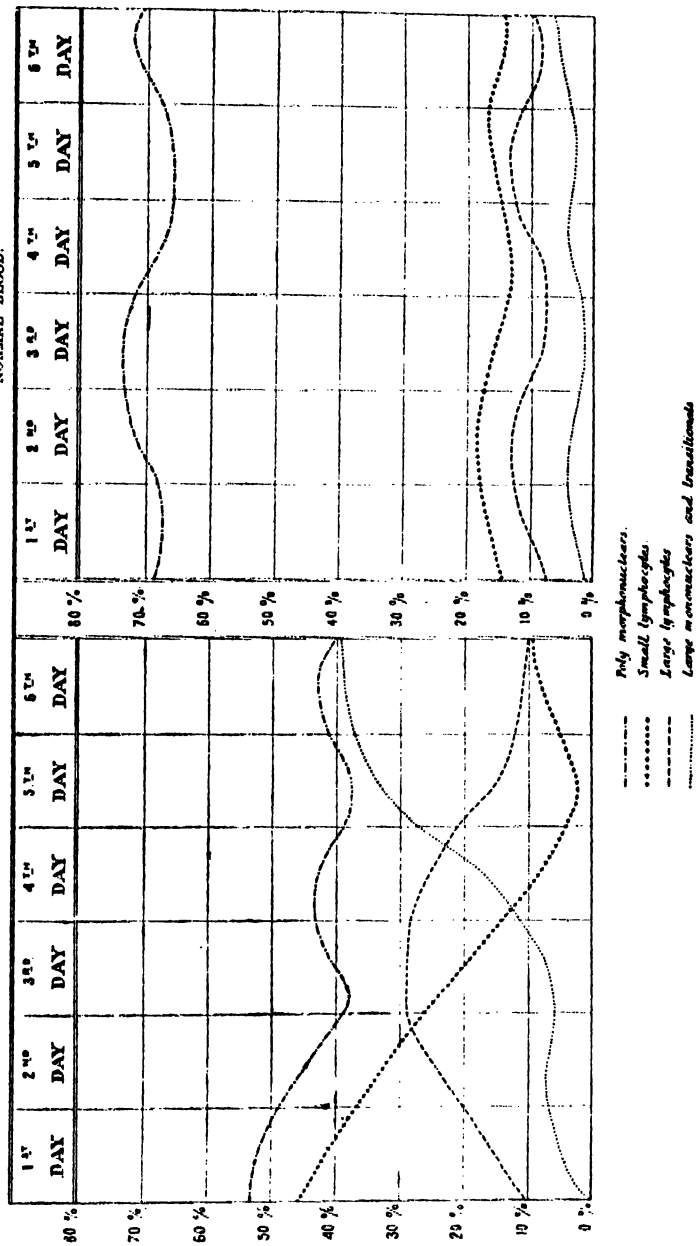


PLATE III.

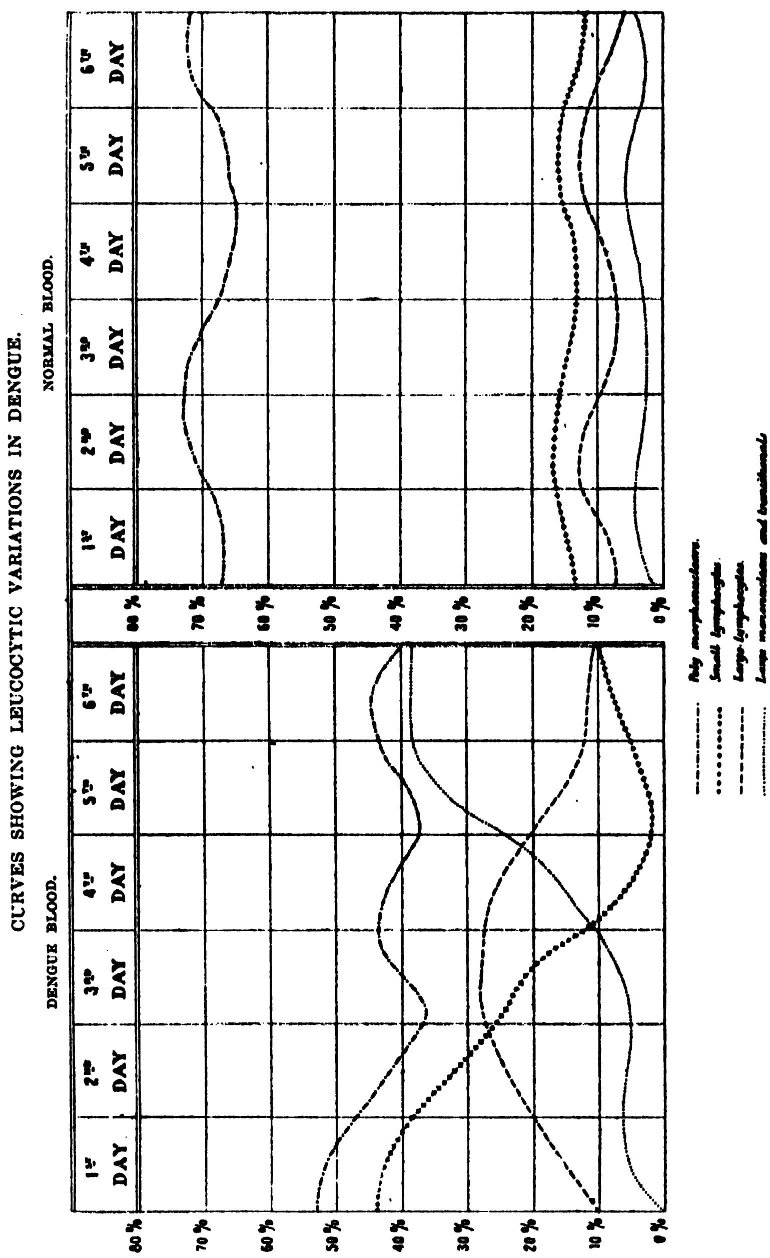

CURVES SHOWING LEUCOCYTIC VARIATIONS IN DENGUE.
DENGUE BLOOD.
NORMAL BLOOD.
1st DAY
2nd DAY
3rd DAY
4th DAY
5th DAY
6th DAY
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %

PLATE IV.

OBSERVATIONS UPON MALARIA: LATENT INFECTION IN NATIVES OF THE PHILIPPINE ISLANDS—INTRACORPUSCULAR CONJUGATION.[1]

By CHARLES F. CRAIG.

(From the Laboratory of the United States Army Hospital, Camp Stotsenberg, P. I.)

INTRODUCTION.

In this contribution it is my purpose to give in brief the results of some observations obtained upon malarial infections while I was on duty at the United States Army post at Camp Stotsenberg, Pampanga, Luzon, P. I.

The locality mentioned is one of the most favorable in the Philippines for the study of malaria, as cases of the disease are numerous in which all varieties of the plasmodium may be observed. The post is situated on the western border of the great Pampangan plain, near the Zambales Mountains, the station proper being placed in the foothills and at a considerable elevation above the plain. The soil is volcanic in nature, and even after the heaviest rains in a few hours becomes perfectly dry. There is no stagnant water within a mile, and therefore there are no breeding places of *Anopheles*. In order to reach the post these insects must travel at least one mile, and as they are at all times numerous it will be seen that in this instance the prevalent idea that they fly but a short distance is disproved. There is a considerable area of jungle country surrounding the station, some of which is situated within a radius of a few hundred yards, but I have failed, after the most careful exploration of these jungles, to find any breeding places of the *Anopheles* nearer to the post than one mile. It was invariably found that when the grass about Stotsenberg was allowed to grow to any great length the mosquitoes to a large extent increased, this being accompanied by a coincident rise in the number of cases of malaria; on the other hand, when it was cut, both mosquitoes and malaria diminished very appreciably.

[1] Read March 1, 1906, at the third annual meeting of the Philippine Islands Medical Association by Charles F. Craig, M. D., First Lieutenant, Assistant Surgeon, U. S. Army, member of U. S. Army Board for the Study of Tropical Diseases in the Philippine Islands.

Mosquitoes of the genus *Anopheles* are at certain times numerous while at others they are not to be found. During the latter portion of the rainy season the *Anopheles* begin to multiply, reaching their maximum during the months of November and December. This coincides with the increase in malarial infections at this post, as is shown in the following table:

Month.	Number of infections.	Month.	Number of infections.
1904.		1905—continued.	
August	24	April	10
September	57	May	14
October	24	June	17
November	58	July	11
December	75	August	29
1905.		September	31
January	76	October	43
February	27	November	54
March	20	December	126

During the five months in which I was stationed at Camp Stotsenberg I observed 386 cases of malaria in the blood of which I was able to demonstrate the parasite. Of these, 248 occurred in Americans and 138 in natives. As regards the type of infection, 98 were infected with the tertian plasmodium, of which 63 were Americans and 35 natives; 8 with the quartan, of which 2 were Americans and 6 natives; and 272 with the æstivo-autumnal plasmodium, 183 being Americans and 89 natives. Of the æstivo-autumnal infections, 258 were due to the tertian æstivo-autumnal plasmodium and 14 to the quotidian variety. There were 8 combined infections with the tertian and the æstivo-autumnal-tertian plasmodium.

The following table illustrates the character of the infections as regards their initial attacks:

Initial attacks, 227; in Americans, 227; in natives, 0.
Recurrent attacks, 38; in Americans, 18; in natives, 20.
Latent infections, 115; all in natives.

From a consideration of the data given above, several points of interest may be noted.

1. In regard to the type of plasmodium present, all varieties of malarial infection occur at Camp Stotsenberg, the æstivo-autumnal ones being the most numerous, the tertian next, and the quartan infections rare.

In the past there has been considerable discussion regarding which types of malarial infection were the most prevalent in the Philippines, and I have always maintained that here, as in almost all tropical regions, the æstivo-autumnal fevers are the commonest and certainly my studies at Camp Stotsenberg have only confirmed my belief. However, it must be admitted that the type of infection present in one locality can not be considered as a criterion in judging of that to be found in another. This is well shown by the fact that at Camp Gregg, 50 miles north of Stotsenberg, the prevailing malarial infections are tertian in character.

Admitting this, I am still of the opinion that the æstivo-autumnal is the prevailing type of malarial infection in the Philippine Islands.

In this connection, it should be remembered that the hyaline forms of the æstivo-autumnal plasmodia are the most difficult of any to demonstrate and therefore they are undoubtedly often overlooked, even by trained observers. Furthermore, it frequently requires repeated examination of the blood and a long and patient search to find them. It is perfectly evident when the data are carefully studied, that many, if not the majority of the so-called "simple continued fevers" which some medical men believe to be so prevalent in these Islands, are really unrecognized cases of æstivo-autumnal malarial infection.

2. Initial attacks of the malarial fevers are most common among the Americans, including in this term all whites. This would be expected, and it is not because of a relative immunity of the natives, for, as will be shown, such a relative immunity does not exist. It is due to the fact that a very large proportion of the natives in this locality are continually infected and suffer from relapses at longer or shorter intervals. It was but rarely that I was able to study an initial attack of malaria occurring in persons of the latter race and then it was only in very young children or in new arrivals.

3. Latent infections are most common in the natives, none being observed in Americans. However, in most of the latent cases a history of previous attacks of malaria could be obtained.

4. Recurrent attacks among the Americans were comparatively rare, due to the fact that every patient infected with malaria when treated in the hospital or in the post was required to take quinine once a week for several weeks and from our experience with this method there can be no doubt of its great efficiency in preventing recurrences of malarial infection.

LATENT INFECTION IN NATIVES OF THE PHILIPPINES.

The subject of latent infection in malarial disease more and more is receiving the attention which it deserves. We now know that an individual may harbor the malarial plasmodia for weeks or even months without suspecting that he is attacked, and during all of this time he may be a source of infection to others.

The observations of Koch, of Stephens and Cristophers in Africa, and of James in India prove that the greatest source of danger to the white man in a malarial locality lies in the native population, especially in the native children. The control of malarial infection in the Tropics will only be possible if quinine is issued to the native, and in the following observations it is my purpose to show that the latter race in the Philippines is the source of malarial infection. I will also attempt to show the futility of endeavoring to rid any locality of malaria so long as the native element in the question is neglected.

By a latent malarial infection we understand one in which the plasmodia of malaria may be demonstrated to be present in the blood of an individual, but in which no clinical symptoms of the disease of sufficient gravity to attract attention are to be observed. The term should not be confined to those instances in which no symptoms of malaria have ever been present, for in recurrent cases between the attacks, the disease is as truly latent as it is before the initial one. Under all circumstances the infected individual obviously is a great source of danger to others.

It appeared probable to me in considering the malarial situation as presented at Camp Stotsenberg, that the natives living in the barrios about the post were in all likelihood the chief source of infection, for while, as has been stated, the sanitary conditions in the post proper were such as to prohibit the belief that malarial infection could originate there, the same could not be said of the native barrios where the breeding places of mosquitoes abounded and insects of the genus *Anopheles* were numerous. In these situations the conditions for the spread of the human infection were ideal. In order to determine how large a percentage of the native population was infected, I made blood examinations whenever they could be obtained of all the natives living within 2 miles of the post. The results proved beyond question that the origin of malarial infection at Camp Stotsenberg is very largely due to the natives living in the immediate vicinity, and that any efforts to limit the disease must take this condition into account. In many of the persons of this race, the clinical symptoms of malaria were present at the time of the examination. Such cases are not included in this paper, which treats solely of latent infection in the native. In a considerable number of the instances of latent infection, even in the youngest children, a history of previous attacks of fever could be obtained, but in none of then were any symptoms of malaria observed at the time of the examination. In all, the blood of 225 natives was examined and 115 or 51.1 per cent were found to be infected.

James, in his work in India, found that the percentage of natives infected varied very greatly in different localities, being less than 5 per cent in some places and as high as 86 per cent in others. The same condition will be found to exist in these Islands and blood examinations of the natives will result in giving valuable data as to the endemicity of malarial disease in different localities in the Philippines. This factor should be considered and such blood examinations of the natives made before permanent military posts or residences are established in the Tropics.

Of the 115 infections found, the tertian plasmodium was present in 29, the quartan in 6, and the æstivo-autumnal in 77; of the latter, 73 were due to the tertian æstivo-autumnal plasmodium and 4 to the quotidian. There were 3 combined infections with the tertian and the tertian æstivo-autumnal plasmodium.

Latent infection in the adult native.—I was unable to examine the blood of more than 45 adults, of which 28, or 62.2 per cent, were infected; of these 5 were due to the tertian plasmodium and 23 to the tertian æstivo-autumnal variety. It is very probable that a further study of the blood of a larger number of adult natives would materially reduce this great percentage of infected cases, but from the above it is evident that the adult Filipino is more often infected than is the negro in Africa or the native of India.

Both Koch in Africa and James in India, have ascertained that the percentage of infected adults is very small. In the planting districts of the Duaro, James stated that he discovered no adults with plasmodia in their blood, although 65 to 75 per cent of the children were found to be infected.

Therefore, it is evident that the adult Filipino possesses little or no relative immunity to malaria, despite the fact that in malarial districts such adults have from childhood suffered repeated attacks of malarial disease. I have notes on several adult natives who, within two years, were admitted to the hospital from 8 to 16 times, suffering from attacks of malarial fever.

Latent infection in native children.—The blood of 180 children was examined, of which 87 or 48.3 per cent showed the presence of malarial plasmodia. Of these 34 were due to the tertian plasmodium, 6 to the quartan one, and 44 to the æstivo-autumnal variety; of the latter, 40 were infected with the tertian æstivo-autumnal and 4 with the quotidian æstivo-autumnal plasmodium. There were 3 cases which had the tertian and the tertian æstivo-autumnal plasmodium combined.

The infections in the children diminished in number with advancing age; thus, between the ages of one month and five years, among 40 children, 79 per cent; of 54 individuals between five and ten years, 37 per cent; and of 53 between ten and fifteen years 24.5 per cent of those examined were infected. These results agree with those of Koch, Stephens and Christophers, James and others, who invariably found that the younger the child, the more susceptible it was to malarial attack.

The following table illustrates the relationship between malarial infection and the age of the individual as observed in native children in the barrios about Camp Stotsenberg; it gives the number of children examined, the number attacked, arranged in five-year periods, the percentage of those infected, and the type of infection:

Age.	Number infected.	Per cent.	Tertian.	Quartan.	Æstivo-autumnal.
1 to 5 years 40	30	72.5	10	4	16
5 to 10 years 54	20	37	8	1	*10*
10 to 15 years 53	13	24.5	5	1	7

*Combined, 1.

Only 146 children are considered in the above table because only in that number could the age be ascertained.

Family infections.—The malarial infections are strictly local, being confined within certain well-defined limits, even in infected localities. Thus Celli, in Italy, observed that the families living upon one side of a canal in a certain town suffered greatly from malaria, while those residing upon the other were practically free. This was very noticeable in the barrios about Stotsenberg, certain places in the barrios being badly infected while others were almost unattacked. Not only was this true, but it was observed that malarial disease was very largely a family one, certain families suffering severely while others were free. The following table illustrates the family character of malarial infection, being compiled from the data obtained in one barrio in which the families resided:

Family.	Number of members.	Number of infections.	Variety.
1	4	2	1, æstivo-autumnal; 1, tertian.
2	3	2	2, æstivo-autumnal.
3	4	2	1, æstivo-autumnal; 1, tertian.
4	5	4	2, æstivo-autumnal; 1, quartan; 1, tertian.
5	4	2	2, æstivo-autumnal.
6	3	2	2, æstivo-autumnal.
7	4	3	1, æstivo-autumnal; 1, tertian.
8	3	2	1, æstivo-autumnal; 1, quartan.
9	3	2	2, tertian.
10	6	4	2, æstivo-autumnal; 2, tertian.

In considering this table, it should be remembered that these infections are latent in character and I have repeatedly observed families in which every member was suffering from a malarial attack, some presenting the symptoms of the disease while in others the condition was latent. Such are Nos. 4 and 10 in the above table. Family No. 4 is of great interest because, of its 5 members, 1 was suffering from a severe attack of tertian malaria at the time I made the blood examinations, while of the other 4, 2 presented æstivo-autumnal, 1 the quartan, and the other the tertian plasmodium in the blood, so that in this one family all the varieties could be studied. From the data which has been given I believe it to be obvious that a large amount of the malaria at Camp Stotsenberg is directly due to the infection of the native, and there is no reason to doubt but that the same is true of every malarial locality in the Philippine Islands. The natives are the culture tubes holding the plasmodia, the mosquitoes the inoculating needles, and the "stranger within the gates" the victim of inoculation. It is also apparent that it is useless to expect to rid a locality, from which the mosquitoes can not be eliminated, of malaria, unless the infection is first stopped among the natives, and in the Tropics, where it is practically impossible to destroy all mosquitoes,

it appears to me that the greatest hope of success in combating malaria lies in the distribution of quinine. It would then make no difference how numerous the *Anopheles* might be, they would be harmless if the native were rendered free from the plasmodium by the use of quinine.

INTRACORPUSCULAR CONJUGATION.

In concluding these observations upon malarial infection I desire to call attention to intracorpuscular conjugation, a phase in the human life cycle of the malarial plasmodia, which I believe to be of the greatest importance, and which makes clear many obscure points in the clinical history of malarial infection.

This process was first mentioned by Mannaberg, but described more at length by Ewing[2] in 1901. Ewing concluded that, while conjugation of the malarial plasmodia undoubtedly occurred in the red blood corpuscles of man, it was by no means constant and probably of no essential importance in the human life cycle of the plasmodia. In a later contribution he states that the process may favor the multiplication of the plasmodia in the human host and that its disappearance tends to limit the malarial infection.

For nearly five years I have devoted much study to this process and have observed it in hundreds of cases. My results are given in detail in a recent publication[3] and at the present time I will only very briefly discuss the subject.

The process of intracorpuscular conjugation, as seen in the malarial plasmodia, is not a sexual act, for no difference in sex can be distinguished in the conjugating bodies. It is a process which is very common in many classes of protozoa and is intended to preserve the reproductive power of the organisms in which it occurs. By continued division, many of the protozoa eventually become exhausted and to prevent this result, conjugation occurs, the two conjugants, which are not sexually differentiated, becoming one, thus bringing about a restoration to former reproductive activity, due to a "rejuvenescence" of the vital activities of the organism. In what way this "rejuvenescence" results we do not know, but there can be no doubt but that if it were not for this interesting process many of the protozoa would cease to exist. In the case of the plasmodia of malaria, conjugation is a phase of the human life cycle and it occurs within the red blood corpuscles. It essentially consists in the permanent union of two hyaline ring forms, or hyaline bodies, is always complete before the development of pigment takes place and it occurs in all the species of malarial plasmodia.

The process may roughly be divided into three stages. In the first one the two hyaline rings are in contact, and in stained specimens it will be

[2] *Journ. of Exper. Med.* (1901), 5, 429; *Clinical Pathology of the Blood* (1903), 454.

[3] Intracorpuscular Conjugation in the Malarial Plasmodia and Its Significance, *Amer. Med.* (1905), 10, 982-1024.

noted that the chromatin of the nuclei in the two parasites is separate and that the union begins in the protoplasm of the organism. In the second stage, which may be designated as that of complete protoplasmic union, the chromatin masses become situated in the protoplasm of one organism which is formed by the union of that from the two. In the third stage, or the one of chromatic union, the two chromatin masses become united and form one large mass situated within the hyaline ring. Sometimes the union of the latter appears to be preceded by the loss of a small particle of chromatin from one of the masses. Briefly stated, intracorpuscular conjugation consists in the union, at some portion of the peripheral protoplasm, of two hyaline plasmodia, the two chromatin granules remaining separate; gradually the protoplasm of the two organisms unites, this union being followed by that of the chromatin masses and of the achromatic substance of the nuclei; conjugation is thus completed, the union being permanent and occurring within the erythrocytes.

The significance of the process.—Intracorpuscular conjugation is present in infections caused by all of the species of malarial plasmodia, namely, in the tertian, quartan, and æstivo-autumnal varieties. In order to understand the significance of the process it is necessary to observe in what type of malarial infection it occurs, whether or no it produces any peculiar clinical manifestations, and whether it is always present or is a rare phenomenon. From my studies, during which I have examined hundreds of specimens of blood from patients suffering from acute initial attacks of malaria, recurrent attacks and latent malarial infections with reference to this process, the following conclusions may be drawn:

First. Intracorpuscular conjugation is invariably present in every acute, initial attack of malarial infection, no matter whether it be due to the tertian, quartan, or æstivo-autumnal plasmodium. It is often difficult to demonstrate conjugating parasites, but repeated examinations will always result successfully.

Second. This process is present in the vast majority of instances of recurrent malarial infection caused by any of the species of malarial plasmodium.

Third. It is almost never present in latent malarial infection, the only cases in which it is noted being those which develop clinical symptoms within a day or two of the time of observation.

Fourth. The process of intracorpuscular conjugation has, in the vast majority of cases, a direct relationship to the severity of the clinical symptoms. When the latter are most severe, the number of conjugating plasmodia is greatest; in acute, initial attacks the conjugating plasmodia are always more numerous than in recurrent ones, unless the latter are of a severe character.

Fifth. The process is most easily observed in pernicious malarial infections, for in such the conjugating plasmodia are most numerous.

Sixth. This form of conjugation is not absolutely essential to sporulation or to the production of clinical symptoms.

Seventh. It is necessary for the maintenance of auto-infection, and its disappearance results eventually in spontaneous recovery of the patient.

As has been stated, conjugation in the protozoa is intended to prevent reproductive degeneration and to maintain the existence of the organism. In the malarial plasmodium the process is without doubt intended to preserve its reproductive power and all the facts which have been observed confirm this opinion.

Malarial plasmodia in man reproduce by spore formation, but a continuation of this process will ultimately end in exhaustion as it does with other protozoan organisms and such exhaustion is prevented by conjugation, after a certain number of generations of the plasmodia has been produced by sporulation. The result is a "rejuvenescence" of the nearly exhausted individuals and renewed activity in sporulation, and thus the malarial infection is maintained. In acute, initial attacks of malaria, conjugating plasmodia are always present, sporulating bodies are numerous and the clinical symptoms are pronounced. In latent infections, the first named are almost always absent, sporulating bodies are of very rare occurrence, and there are no clinical symptoms of malarial infection. During spontaneous recovery, conjugating plasmodia entirely disappear and if a relapse occurs they only reappear just before the occurrence of clinical symptoms. All of these facts point to but one conclusion, namely, that intracorpuscular conjugation favors the reproduction of the plasmodia of malaria, plays a very important part in the appearance of the clinical symptoms because it favors sporulation and its disappearance is the principal cause of spontaneous recovery.

TROPICAL FEBRILE SPLENOMEGALY.[1]

By Paul G. Woolley.

(*From the Serum Laboratory, Bureau of Science.*)

The disease known as tropical splenomegaly is characterized chiefly by splenic hypertrophy, emaciation, an irregular temperature uninfluenced by quinine and certain gastro-intestinal symptoms such as diarrhœa or dysentery. Christophers also maintains that amœbæ are invariably present. Such cases have been reported from various parts of India, from Egypt, China, Tunis, Algiers, Arabia, and the Philippine Islands.

Giles first considered the disease as a combination of malaria and anchylostomiasis and Bently thought it to be due to infection with *M. melitensis*. Rogers at first supposed it to be the result of a severe chronic malarial infection, but later in the cases from all of the above-mentioned sources save the last, a peculiar parasite has been described, first by Leishman in a case at Dum Dum in India, later by Donovan, Christophers, Rogers, Manson, Low, Phillips, Marchand, Ledingham, and others.

Since the publications of Leishman and Donovan, much speculation has arisen regarding the place which, in our classification, should be assigned to these parasites. Ross thought them to be an involution form of trypanosoma, and Laveran concluded that they belonged to the genus piroplasma or pirogma. Late investigations seem to point to the wisdom of Ross's conclusion, the weightiest reason being that Rogers has stated that in cultures of the bodies obtained by splenic puncture, after some days he has been able to demonstrate unmistakable trypanosomata which, to be sure, were very immature in most cases. Through the kindness of Dr. Strong I have had the pleasure of seeing some of Rogers's preparations, which were smears both from the spleen pulp and from cultures and while to judge from these it is not clear just what the relation of the two preparations is, nevertheless, in the cultures there were structures which very closely simulated trypanosomata. The Leishman bodies themselves were not especially convincing to me, perhaps because I have seen too few. However that may be, there seems to be a

[1] Read at the third annual meeting of the Philippine Islands Medical Association, March 2, 1906.

concensus of opinion in considering these bodies to be the causative parasites of *kala-azar*.

When present, these parasites are found in large numbers. They are difficult to distinguish in fresh specimens, but when seen can be differentiated from the platelets by their greater refractive power. They are most easily stained by the Romanowsky method or one of its modifications, and under these conditions their diameter is from 1.5 to 3.5 μ. They are usually circular or oval in form and often have a cockleshell appearance. They usually show two chromatin masses, a larger one which stains faintly and a small one which takes on an intense color. The former often is rod-shaped and the latter rounded, bilobed, or heart-shaped. They have been observed in the spleen, in intestinal ulcers, in the liver, mesenteric glands, bone marrow, kidneys, and in the skin in the disease known as Oriental sore.

It was in view of these facts and in the hope of being able to study these parasites that Dr. Musgrave, Dr. Wherry, and I began carefully to examine all cases of splenomegaly which we could find and control. The results of this study were published early in this year in the Bulletin of the Johns Hopkins Hospital.[2] In this paper we stated that splenomegaly was not infrequent in Filipinos, and that it was not difficult to find types corresponding with those of Osler (*Brühle*), Banti, and others. We then proceeded to describe some cases which apparently should be classed with the group of tropical, febrile splenomegaly.

These cases were characterized by the following symptoms: Splenomegaly, rheumatic pains, edema, diarrhœa with or without hepatic enlargement, and remittent fever. In none of these could malaria be found, neither was the course of the disease influenced by quinine, in none were the leucocytes above 8,000 (they averaged about 6,300), and in but one was the number of red corpuscles less than 3,000,000 per cubic millimeter. Unfortunately, at the time we made our observations we had no accurate means of determining the hæmoglobin, of which we have no record; however, it may be suspected from the appearance that there was some diminution. In five cases, splenic puncture was undertaken and in four of them examination in smears and cultures, and inoculation into guinea pigs and monkeys gave no result. In the fifth one there appeared in glucose-ascitic agar a faint growth of a small, coccus-like organism, which resisted transplantation.

In one of the seven cases which we reported the eosinophiles varied from 25.5 to 43 per cent, in another from 8 to 11.5 per cent; generally, the mononuclears were increased. The duration of the disease at the time of the last notes which we took was from three to fifteen years.

But one case has come to autopsy. In this instance the chief lesion consisted in a congestion, hypertrophy, and fibrosis of the spleen. Since the report of these cases another very interesting one has been brought to my attention, and for the opportunity of reporting it I have to thank Dr. Musgrave, from whose services in St. Paul's Hospital, Manila, it came.

[2] *Bull. Johns Hopkins Hospital* (1906), **17**, 28.

The patient was a Filipino *banquero*, aged 26, a native of Rizal Province. There was a past history of beriberi. He was admitted to St. Paul's Hospital suffering from ascites and splenomegaly, a condition which he said had been present for two months. He had an irregular temperature and a slight diarrhœa. (See chart No. 1.) His appetite was good. Blood examinations from time to time showed no malarial organisms, nor had quinine any effect upon his temperature. His urine showed a trace of albumen, and hyaline and granular casts. The stool examination was negative for parasites. Blood counts showed: Hæmoglobin, 80 per cent; red blood cells, 4,264,000; leucocytes, 5,400. A differential count showed polymorphonuclear leucocytes, 74 per cent; large morphonuclears, 10 per cent; small morphonuclears, 2 per cent; eosin, 11 per cent; cells, 3 per cent. From the time of his entrance into the hospital he had an irregular, remittent and intermittent temperature. He died a month after entrance. The clinical diagnosis was Banti's disease. Two days before his death a splenic puncture was undertaken and smears and cultures were made with the citrated material which was obtained. The smears showed a very small, polar-staining, rod-shaped or diplococcoid organism which was present in small numbers. The culture in glucose broth (+1 to phenolphthaleïn) after two days produced a powdery growth in the bottom of the tubes and a slight diffuse cloudiness. Agar cultures gave no growth. The autopsy was performed six hours after death.

Rigor mortis was present in the legs, feet, and jaws, but not in the anus. The pupils were normal. The lymphatic glands of the groins and axillæ were slightly enlarged and firm. Over the area of splenic dullness, a few centimeters from the midabdominal line and about a hand's-width above the pubis, was the puncture wound caused by the aspirating needle. The body was poorly nourished, the mucous membranes pale and slightly jaundiced. There were some pigmented scars on the shins, as there are in nearly all laboring Filipinos, but there were no periosteal irregularities. After opening the body, the point of entrance of the needle into the spleen could barely be seen, and about it there was no evidence of hæmorrhage or inflammation. The omentum was curled up along the transverse colon. The peritoneum was slightly dull in appearance and showed some points of injection. In the pelvis and flanks there was from 200 to 300 cubic centimeters of blood-stained fluid. The appendix apparently was normal. The left pleural cavity was obliterated by old adhesions. The right was normal. Both lungs were somewhat edematous and the seat of moderate hypostatic congestion.

The pericardium contained about 100 cubic centimeters of a clear, amber-like fluid. There was one large milk spot on the anterior surface of the heart. The myocardium was flabby and cloudy. The epicardial fat was somewhat increased in amount and along the auriculo-ventricular groove was a series of petechiæ. The cardiac valves apparently were normal. The auricles were filled with goose-fat clots. The liver weighed 750 grams and was very firm and resistant. Its surface was covered with nodular elevations varying in size from 1 to 5 millimeters. These elevations were of a greenish-yellow color in the center and were surrounded by zones of congestion. The cut section was granular and mottled yellowish-green and brownish. The increase of fibrous tissue, easily to be seen with the unaided eye, was diffuse. The gall bladder was small and contained no stones. The spleen weighed 1,770 grams and was firm in consistency. Its surface was smooth. It cut with increased resistance and the cut surface showed nó tendency to diffluence. The increase in fibrous tissue was evident to the naked eye. The malpighian follicles were not noticeable. The kidneys were somewhat enlarged, pale and very flabby. The capsules were easily removed. The intestine contained liquid fæces with uncinaria and trichuris. In the large

intestine there were a few quite small, but rather ragged, superficial ulcers, surrounded by dark, pigmented zones. The submucosæ was œdematous and injected. The mucous membrane of the large bowel as a whole was somewhat œdematous and injected, that of the small bowel showed no change other than a catarrhal one. The mesenteric, intraperitoneal, and internal inguinal glands were markedly enlarged, and were, as a rule, pale, but with areas of congestion. The great blood vessels showed no abnormality. The pancreas was firm and pale.

Anatomic diagnosis.—Splenic hypertrophy; atrophic, hepatic cirrhosis; subacute, parenchymatous nephritis; abdominal, lymphatic, glandular hypertrophy; hypostatic congestion of the lungs; petechiæ beneath the visceral pericardium; persistent thymus.

At autopsy, cultures and smears were made from the spleen, liver, and heart. Those from the heart and liver showed no organisms. Those from the spleen gave a considerable number of small bacilli, similar to those found in the antemortem preparation. Cultures on glycerine, agar, and blood serum after forty-eight hours showed numerous discrete, minute, translucent, moist colonies. Transplants were made on various media with the following results: On alkaline and acid agar (1 per cent to phenolphthaleïn), glycerine, glucose and lactose, agar and blood serum, the organism in twenty-four to thirty-six hours gave rise to fine, almost transparent, moist columns, which increased but very little in size during the four or five days during which they remained alive. In glucose, saccharose, and inulin bouillon the growth was scarcely perceptible and took the form of a firm, flocculent precipitate, in which the organisms rapidly died out. Milk was acidified within twenty-four hours, coagulated in seventy-two hours, the caseïn separating from the whey in six days. On potato, the growth was not visible to the eye, but nevertheless it was present. The organism thrived best and lived longest in milk and in potato. It did not form indol. This organism, stained by Gram's method, is non-motile and pleomorphic, but when young and vigorous it appears as a very small, polar-staining rod. It ferments all of the sugars to a slight extent, without gas formation This was indicated by a change in acidity, which in the case of glucose required 0.2 cubic centimeter N/20 NaOH, in that of lactose 0.1 cubic centimeter, with saccharose 0.2 cubic centimeter, and inulin 0.25 cubic centimeter per cubic centimeter of the culture media, to neutralize. (The titrations were performed with phenolphthaleïn as an indicator.)

A monkey (*Macacus cynomologus*) was inoculated subcutaneously with 1 cubic centimeter of a suspension of the organism from an agar culture seventy-two hours old. A second monkey was inoculated in the same manner with 1 cubic centimeter of a suspension from a seventy-two-hour blood serum culture. The results, as far as the temperature was concerned, are shown by Charts Nos. 2 and 5. The organism used in the case of monkey No. 4 had been grown in blood serum for six generations. The constitutional symptoms were most marked in No. 1, which lost its appetite completely after a few days. This monkey was killed on the seventh day after inoculation. The spleen was not enlarged and the other organs showed no microscopic changes. Cultures from the blood and spleen were made upon agar, bouillon (—1 to phenolphthaleïn), and blood serum. No growth occurred in those from the spleen, but in the ones from the blood upon agar and blood serum a slight one occurred

in the water of condensation. One cubic centimeter of blood from this monkey was inoculated into another one (Chart No. 3), and 1 cubic centimeter of an extract of spleen pulp into a third (Chart No. 4). In monkey No. 1 the liver was much congested and there was a marked congestion at the site of inoculation. One cubic centimeter of a pure blood serum culture seventy-two hours old was also inoculated into a rabbit. No effect was produced and the animal is still alive and active. As the charts will show, monkey No. 4 died a month after inoculation, during which time it has had a very irregular temperature. Unfortunately, death occurred late in the afternoon and the animal was badly infected (post-mortem) when it was examined. The organisms with which it had been infected could not be isolated. The spleen was not especially enlarged. The other monkeys (Nos. 2 and 3) are still alive and will be closely watched.

Pieces of the tissues obtained at autopsy from the original case and fixed in Zenker's solution were imbedded in paraffin and sectioned. Sections were stained with carbol-fuchsin-gram-picro-indigo carmin, methylene blue and eosin, hæmatoxylin and eosin, resorcin fuchsin, Mallory's reticulum stain, and by Gram's method. The liver showed the usual picture of a well-marked mono- and multi-lobular cirrhosis with a slight degree of central cirrhosis. No bacteria could be demonstrated. The spleen section showed a marked congestion and a moderate degree of fibrosis which was especially noticeable in the immediate vicinity of the blood vessels.' Atrophy of the malpighian bodies was also present. Sections stained by Gram's method showed clumps of small, polar-staining rods, scattered irregularly in the organ but chiefly noticeable in the more congested parts. None of these organisms were demonstrated either in the follicles or in the lymph glands.

If we compare these cases with those reported as *kala-azar* we see that, aside from the one point of absence of the Leishman bodies, a close analogy exists between them. Diarrhœa is a common feature in all of them. While, perhaps, amœbæ do not play a part in the causation of this symptom, yet, in the Tropics, other intestinal parasites are so frequent that we rarely encounter a native's stool free from one variety or another and frequently several may be met with simultaneously. In addition to the diarrhœa, the symptoms common to all of our cases were splenic enlargement, irregular temperature, transient œdemas, rheumatic pains, loss of weight and strength, and probably anæmia. Together with these, the liver may be enlarged or atrophic and there may be ascites. Of all of these symptoms the most important is splenomegaly. What is its cause?

In the Philippine Islands and elsewhere in the Tropics, malaria is an extremely common affection and its occurrence might explain the increase in the size of the spleen. However, in sections we do not find the evidence we should expect, for we encounter neither pigment nor parasites. Then, too, the irregular temperature in splenomegaly is not satisfactorily explained on these grounds, for quinine does not affect it. Another disease which we are beginning to consider a much more common

one than formerly is syphilis. But the assumption that the latter is present does not explain the temperature, for this is uninfluenced by mercury.

There remains the possibility of a chronic infection or intoxication originating in the intestinal tract. As we have seen, the bowel when symptoms of diarrhoea are present, in almost all instances is considerably damaged. In the last case described above, there were well-marked ulcers and in this and in others, trichuris and uncinaria occur. Is it not possible that the progressive cirrhosis of the spleen and liver is the result of continued absorption from the intestinal tract, aided by the increased permeability of the latter? It may be possible that the continuous absorption of toxins causes the cirrhosis and the penetration of bacteria produces the temperature variation. However that may be, it seems reasonable to assume that the condition may be explained upon the basis of intestinal absorption and that the condition of the intestinal walls may be the factor which determines the presence of a febrile or afebrile splenomegaly.

One striking feature of the symptom complex in the cases which we have examined is the sudden onset of the fever which resembles that of malaria or dengue.

I still believe, as I did after studying the first seven cases, that although a certain number of cases of tropical, febrile splenomegaly may be due to infection with the Leishman body, it will be necessary to search for further etiologic data before we can understand and satisfactorily classify the whole group of cases which are characterized by the general gross features of *kala-azar*, and I believe in all probability that there will be found associated with the clinical picture various organisms and that the symptoms will depend chiefly upon intestinal conditions and pathologic changes in the intestinal walls.

ILLUSTRATIONS.

[Photomicrographs by Martin.]

PLATE I.

FIG. 1. Section of spleen stained by Gram's method showing a focus of the organisms described. X 1000.

2. Same as fig. 1. X 1920.

PLATE II.

FIG. 1. The bacillus from a culture on glycerine agar, Gram's stain. X 1920.

2. The same organism, Gram's stain from bouillon culture. X 1920.

PLATE III.

FIG. 1. The same grown on potato during sixty hours. X 1920.

2. The same grown on blood serum ninety-six hours old. X 1920.

FIG. 1.

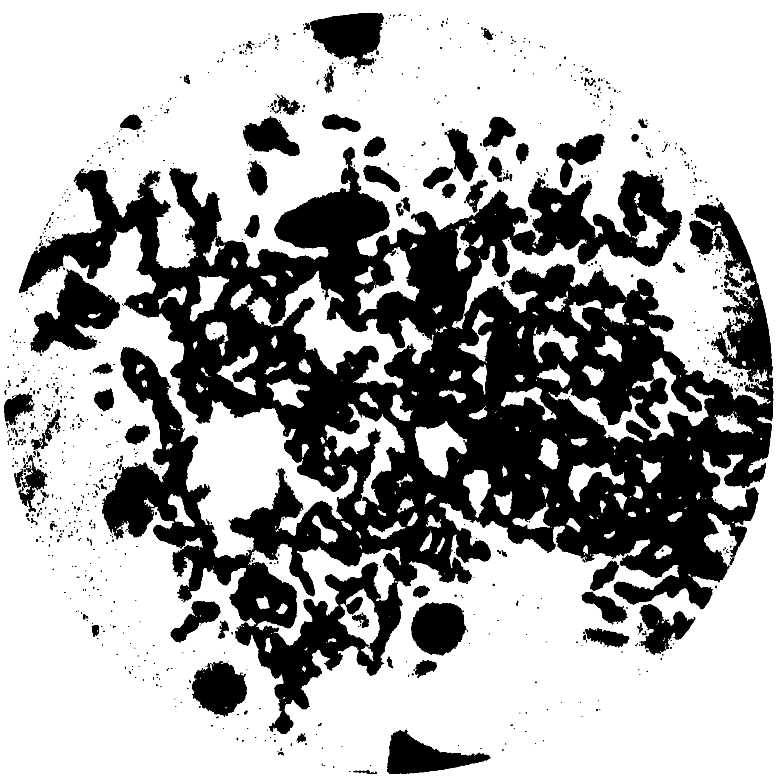


FIG. 2.

PLATE I.

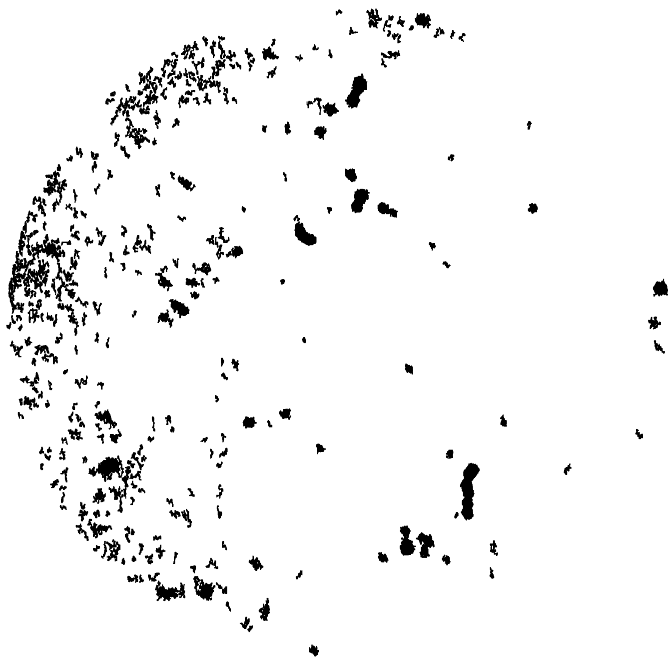


FIG. 1

FIG. 2

PLATE II.

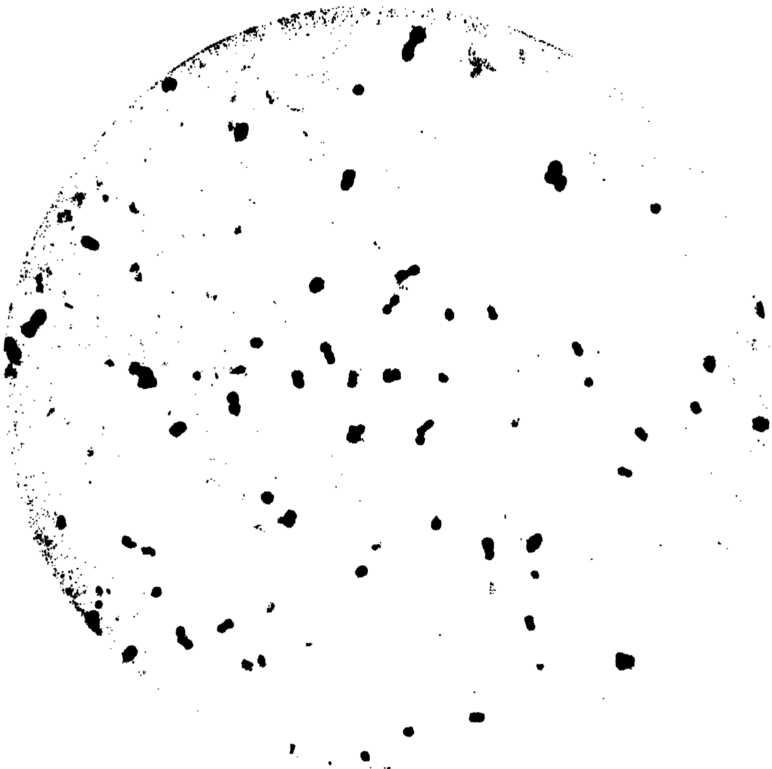
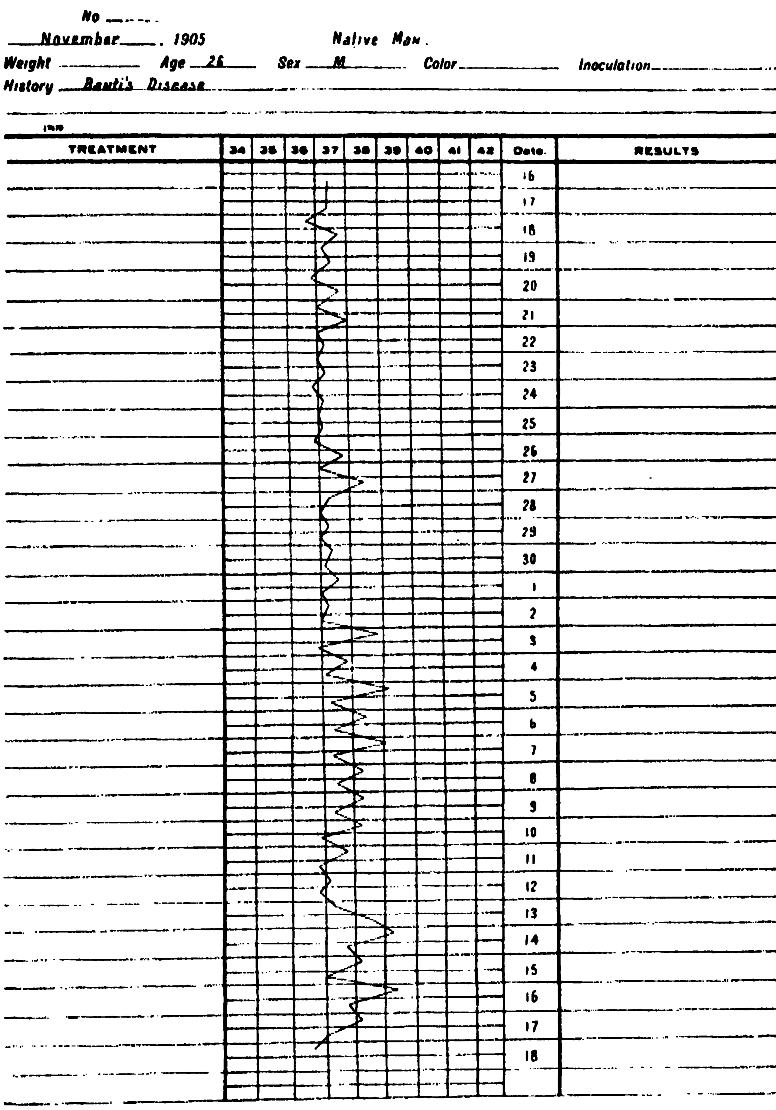


FIG. 1.

FIG. 2.

PLATE III.


No
November. 1905
Native Man.
Weight
Age 26
Sex M
Color
Inoculation
History Banti's Disease
TREATMENT
34
35
36
37
38
39
40
41
42
Date.
RESULTS
16
17
18
19
20
21
22
23
24
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1
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16
17
18

CHART NO. 1.

Monkey No. 1

December ______, 1905

Weight ______ Age ______ Sex ______ Color ______ Inoculation ______

History Inoculated with 1 c.c. suspension of bacillus Case of Banti's Disease

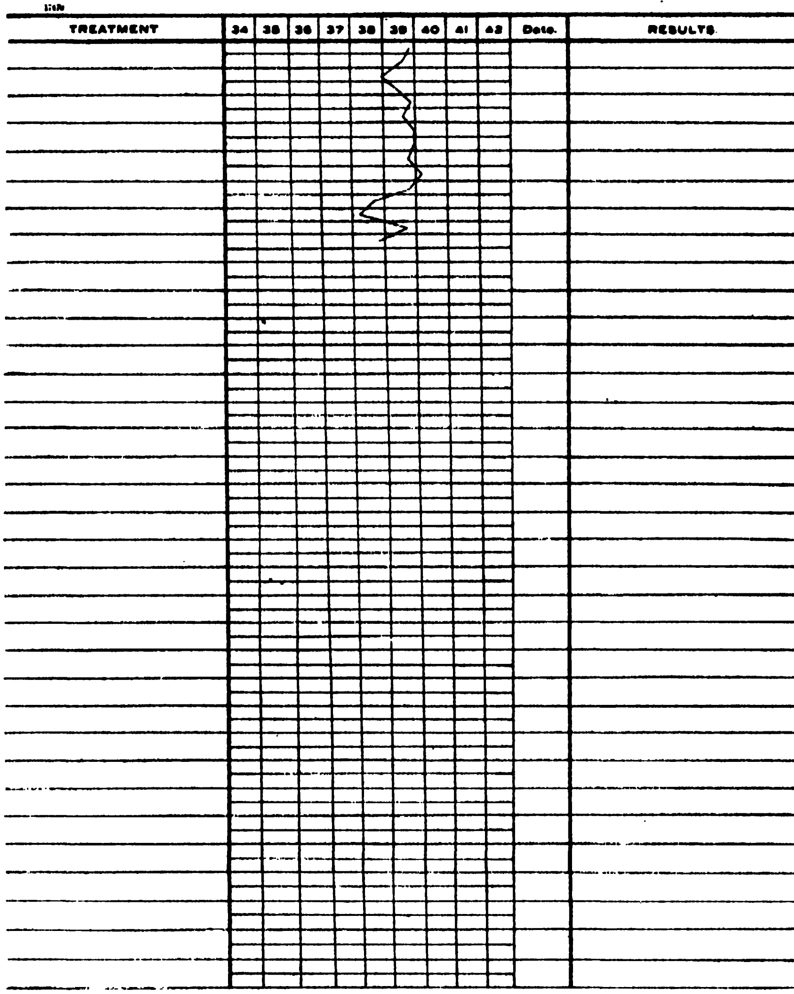

CHART No. 2.

Monkey No 2

January 1906

Weight Age Sex Color Inoculation

History Inoculated with 1 c.c. Hearts blood from Monkey "1

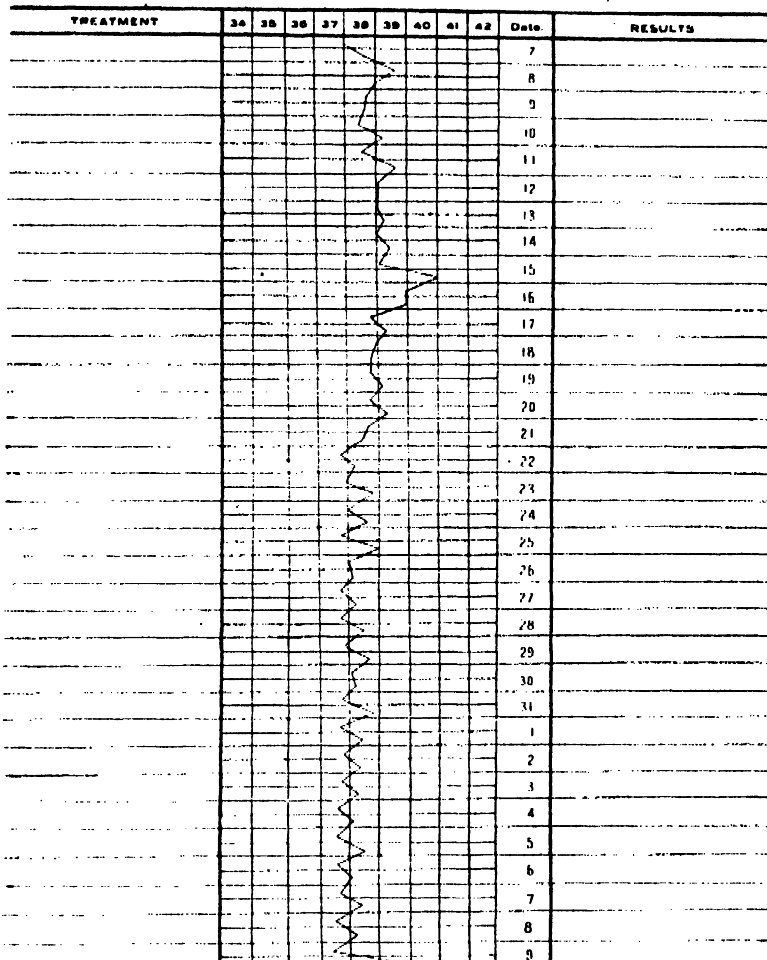

CHART No. 3.

Monkey No. 3

January, *1906*

Weight ______ *Age* ______ *Sex* ______ *Color* ______ *Inoculation* ______

History Inoculated with 1 c.c. splenic pulp Emulsion Monkey °1

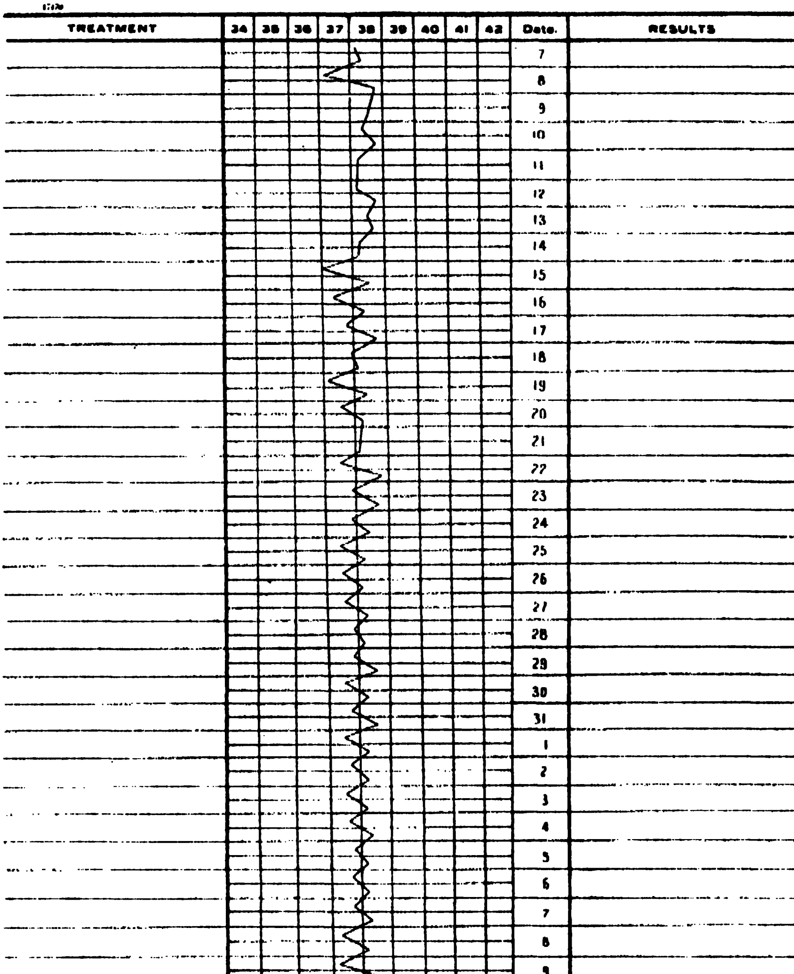

CHART No. 4.

Monkey No. 4

January, 1906

Weight ——— Age ——— Sex ——— Color ——— Inoculation ———

History Inoculated with 1 c.c. 72 hr. Blood serum culture in suspension

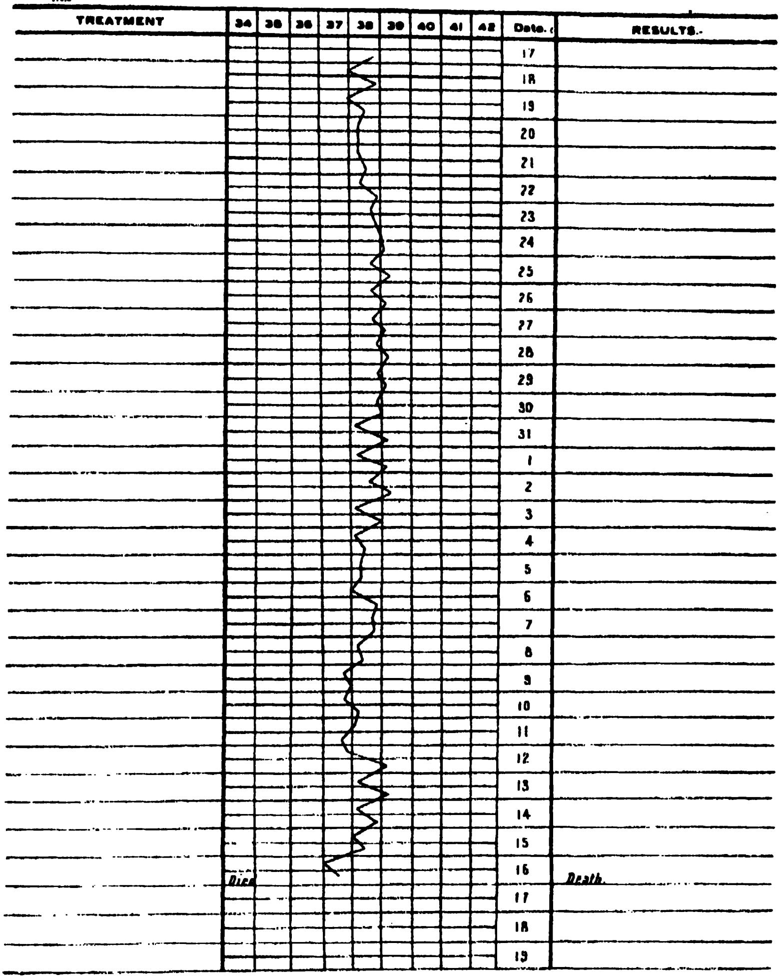

CHART No. 5.

42210——8

AMOEBIASIS: ITS ASSOCIATION WITH OTHER DISEASES, ITS COMPLICATIONS, AND ITS AFTER EFFECTS.[1]

By W. E. Musgrave.

(*From the Biological Laboratory, Bureau of Science.*)

In this paper the more established facts concerning this subject will briefly be described, attention will be paid to points more or less neglected in previous publications and finally, certain new observations tending to elucidate some of the problems will be added. The subject may be outlined under the following headings:

I. Diseases and conditions accidentally associated with amœbiasis which do not modify the course of either infection.

II. Diseases of different etiology in which the association produces decided modifications in one or both.

III. Direct complications.

(*a*) Of amœbic origin.

(*b*) Of other parasitic or of bacterial origin.

IV. After effects of amœbic diseases.

I.

Complete discussion of the first heading would practically include the whole subject of tropical medicine, because amœbiasis in the Tropics is such a very common affection that almost every other disease has, at different times, been found associated with it.

Pregnancy, because of its frequent association with amœbiasis in females, is the only condition which will receive notice in this connection. Local treatment of the dysentery often increases the vomiting of pregnancy, but the danger of abortion from this cause is even less than that to which the untreated amœbiasis itself gives rise. Experience has taught us to give pregnant patients suffering from amœbic colitis the same thorough irrigations which are with confidence applied in other non-pregnant cases afflicted with this disease.

[1] Read March 3, 1906, at the third annual meeting of the Philippine Islands Medical Association.

II.

The second heading is of much greater importance and includes such diseases as malaria, beri-beri, Bright's disease, specific bacterial dysentery, tuberculosis, chronic rheumatism, certain nervous affections, and other diseases, some of which will also be duly considered under the third and fourth headings.

MALARIA.—This is far the most important disease in this second class. True malarial dysentery is recognized by many writers, but, entirely apart from this, the association of a simple, malarial infection with amœbiasis is very frequent, and the combination of the two makes each disease much more refractory to treatment than is either one alone. The symptomatology is also much modified and, in cases in which, in addition, liver abscess is suspected, the diagnosis is often very difficult. In these combined infections of malaria and dysentery, the treatment of the former affection alone often causes an improvement of the amœbic disease of the bowel, and quinine irrigations usually give satisfactory results in both, although obviously, in severe cases of malaria, the administration of larger doses of quinine is also advisable.

BERIBERI is endemic in the Philippine Islands and is frequently associated with amœbic and other parasitic infections of the intestine. Without questioning a specific, infectious etiology for beriberi, I would call particular attention to the favorable influence which treatment directed to the removal of intestinal parasites often has upon the course of the co-existent neuritis. Beriberi also exerts a bad influence on the amœbic infection, and if, as is claimed by some writers, changes are necessary in the intestinal wall before amœbæ can produce lesions, clinical and pathological observation would suggest beriberi as being one of the diseases in which such primary changes of the intestine would occur. M. Herzog, of this Bureau, has also called attention to this point in a paper read before the Manila Medical Society, February 8, 1906.

BRIGHT'S DISEASE.—Marked disturbance of kidney function, and even organic changes therein, may directly complicate the amœbic infection, and these will be discussed under the third heading, but, apart from this, there is the rather frequent association of established Bright's disease (of alcoholic or other etiology) with amœbiasis. Such a conjunction is a particularly difficult one to treat, because of the increased intensity of both diseases when combined, above that which would be expected from either alone. This increase becomes very pronounced in uræmia.

III. DIRECT COMPLICATIONS.

LIVER ABSCESS.—Amœbic abscess of the liver is of rather frequent occurrence in the Tropics and subtropics and is encountered sporadically in nearly all parts of the world. More than 95 per cent of all liver abscesses seen in the Philippine Islands are of this variety.

Frequency and association with intestinal amœbiasis.—Rogers collected 200 cases of amœbic liver abscess in examining 5,000 miscellaneous autopsy records in India. Among 10,603 patients (from American troops) in the Philippine Division Hospital it was diagnosed 34 times (Collin). Strong and Musgrave encountered liver abscess 27 times in 400 miscellaneous autopsies performed, for the greater part, on American soldiers in the Philippines. In the first thousand patients, from a cosmopolitan population, admitted to St. Paul's Hospital, Manila, liver abscess was found in 12.

In the great majority of patients the abscess complicates an amœbic infection of the colon *and its frequency in this disease* is variously estimated as being from 5 to over 75 per cent of the cases. Councilman and Lafleur collected 486 liver abscess cases from the records of 2,430 autopsies on dysentery. Rogers found it in about 20 per cent of his large series; Strong and Musgrave in 23 of 100 fatal cases, mostly among American soldiers; Harris in 15 of 95; Futcher, in 27 of the 119 in the Johns Hopkins Hospital series; Craig in over 50 per cent of 3,210 collected cases and in 33.7 per cent of his own series; Collin in 34 of 859 among American soldiers.

In the first 100 cases of amœbiasis treated in St. Paul's Hospital, of which 9 came to autopsy, it has been found 12 times. The diagnosis in each instance was made during life. It occurred 10 times in Americans and Europeans and twice in natives of the Philippine Islands.

In the great majority of cases the evidence that the abscess is secondary to amœbic infection of the colon is conclusive, but there are a number of instances in which it might appear to represent a primary amœbic infection of the liver, since the most careful search at autopsy fails to show any evidence whatever of a lesion of the bowel. Amœbic liver abscess is considered to be a distinct disease. That amœbæ could reach the liver in any other way than from the bowel seems improbable, and, if we admit this, there are then but three possible explanations of the *apparently* primary infection of the liver:

First. Amœbæ may reach the liver by the gall ducts.

Second. They may penetrate the bowel wall without producing apparent lesions; or

Third. The bowel lesions may have been entirely repaired.

The first of these propositions is untenable, because amœbæ have not been found in the gall bladder and ducts or even in the upper part of the intestine and, moreover, the undiluted bile is, at least in cultures, toxic for amœbæ. The second is a possible one, especially when we consider Schaudin's observations and the pathology of the early lesions of colon infection, as pointed out by several observers, and recently by Woolley and Musgrave.[2] However, the third furnishes the most satisfactory explanation, for it is well known that the process of repair, even in very extensive lesions of the intestine, is often so complete that no macroscopic evidence of the disease remains. In whatever manner the mode of entrance may be explained, the fact is fully established that amœbic abscess of the liver

[2] *Publications Biological Laboratory, Bureau of Government Laboratories*, Manila (1905), **32**, 31. Also *Jr. A. M. A.* (1905), **45**, Sept. 16 and Nov. 4.

may exist without *discoverable* lesions in the bowel *at the time of the death of the patient.*

Since my previous discussion which touched this subject,[3] one case of liver abscess has been encountered, in which the most careful and persistent search failed to give evidence of a previous dysentery, and there was no history of antecedent dysentery or diarrhœa. However, similar conditions and findings have occurred in other cases in which, some months before, there was a perfectly clear history of dysentery. Therefore, by taking everything into consideration, the conclusion is justifiable that the majority, if not all, of the cases of amœbic abscess of the liver in which, at autopsy, intestinal lesions were not discernible, occurred in individuals who had previously suffered with amœbic colitis, which had finally healed, and in which the amœbæ reached the liver during the period of active ulceration of the colon.

Relation of liver abscesses to the location of intestinal ulcers.—There does not appear to be any definite relation between the occurrence of liver abscess and the location of the intestinal lesion, or between it and the location of an intestinal perforation, should one occur, although the observations of several recent writers and my own indicate liver abscesses to be most frequent where there is ulceration of the cæcum and ascending colon.

In one series of 27 abscesses, 15 occurred without the perforation of the ulcers in the intestine; there were ulcerations in the cæcum and ascending colon in 8; in 1 in the transverse, and in 3 in the descending colon.

Again, in another series, we have perforations in the cæcum or ascending colon 9 times with 6 cases of liver abscess, and perforations in the descending colon 8 times with 1 liver abscess. In one series of 13 perforations of the intestinal ulcers, without liver abscess, 10 were below the hepatic flexure of the colon. In 11 solitary abscesses of the right lobe, the colon was ulcerated throughout in 6, and the cæcum and ascending colon alone in 5. In 2 multiple abscesses of the right lobe, the intestinal ulceration was general; in 8 multiple ones of right and left lobes, the entire bowel was ulcerated, and in 1 solitary abscess of the left lung, the transverse colon alone was found, at autopsy, to be ulcerated.

The location and the number of abscesses.—In over 90 per cent of all amœbic abscesses the right lobe is involved, either alone or in connection with other portions of the organ. It is the only one affected in over 70 per cent; and in 40 to 65 per cent of all cases the abscess is a solitary one. The most common condition by far, is a solitary abscess situated in the dome of the right lobe, and in this connection it must be remembered that solitary abscesses are not infrequent in the left lobe, less common in the *lobus spigelii* and that they occur only occasionally in the quadrate lobe. In rare instances, also, multiple abscesses have been confined to the left lobe, and once I have seen three small amœbic ones in the *lobus*

[3] *Publications Biological Laboratory, Bureau of Government Laboratories,* Manila (1904), 18, 112.

spigelii alone. Although small abscesses are frequent and larger, solitary ones are occasionally seen in the deep substance of the liver, in the great majority of cases they are rather close to the surface, whichever lobe is involved. Sometimes, shallow abscesses several centimeters in diameter are situated between the dome of the liver and the diaphragm, but not extending more than 1 or 2 centimeters into the liver substance, the superior surface of such abscesses being entirely formed of a portion of the diaphragm. Twice I have seen very shallow, subcapsular abscesses of from 4 to 9 centimeters in diameter in other portions of the liver, one being situated on the inferior surface. Liver abscesses may vary in size from those having a diameter of 1 millimeter or less to the ones which fill almost the entire, involved lobe. My records include one in which the right lobe was only a shell, containing, at autopsy, over 4 liters of fluid.

Predisposing causes.—Age is a very important factor. It is true that amœbic abscesses have been reported in *children;* Amberg collected 35 cases of amœbic infection in individuals of this class, with 5 liver abscesses, and there have been several later reports confirming these results, but, at least in the Philippine Islands, it is of very rare occurrence. I have not encountered a case among a large number of children under 10 years of age who have been treated for intestinal amœbiasis, nor has one been found during several years of general post-mortem work in this country. Old age also seems to be somewhat protective, for my records show only one case in a man over 60 years of age. The disease is by far the most frequent in young adults and these records bear out the usual statement that males are much more often attacked than females.

Race seems to play an important part in the frequency of this disease. Several writers have noted the comparative infrequency of liver abscess in natives of the Tropics, and this is emphasized by the conditions in Manila. I have not seen an amœbic abscess in a Filipino. However, a few such cases have been observed by J. R. McDill, R. P. Strong, M. Herzog, M. T. Clegg and others in Manila. Intestinal amœbiasis is *very* prevalent among Filipinos.

The Chinese, owing, no doubt, to their excellent care in food and drink, are rarely sufferers from intestinal amœbiasis and, with my limited material, no case of abscess of the liver has been found among them.

Alcohol, in my opinion, has been given an overprominent place as a predisposing cause of liver abscess and although my statistics are not conclusive in this respect, it is certain that many cases are encountered among individuals who, without doubt, are non-alcoholics. The unacclimated are particularly liable to develop the disease and it is believed all will concede that very few persons drink less in their home countries and during the early days of their stay in the Philippines than they do later on.

The physical condition of the patient, the presence of associated disease, particularly of malaria, of the anæmias, and of other affections which are attended with intestinal autointoxication and with liver changes, naturally suggest themselves as predisposing factors, but definite and conclusive data are very difficult to obtain.

There are also other influences which are not fully understood and which may play a part as predisposing factors. Among these we must not lose sight of the condition of and the changes in the liver itself previous to infection. It may be stated without going more fully into the subject at this time that cultural experiments with amœbæ, now being carried on in this laboratory, indicate that amœbic abscess rarely or never develops in an otherwise normal liver, notwithstanding the fact that amœbæ may be almost constantly poured into it from an ulcerated bowel. It has already been pointed out in a previous paper that rational treatment has brought about a great relative decrease in the frequency of liver abscess as a complication of the intestinal infection.

The way in which amœbæ and bacteria reach the liver, and the manner of abscess formation have been a source of much discussion and are still in doubt. In the case of multiple abscesses, the evidence is fairly conclusive that the portal circulation acts as the means of transmission of the infection and indeed, no other explanation is at all satisfactory. No doubt this is also the principal channel by which the amœbæ are transferred when a single abscess manifests itself, but there are also valid reasons for believing that the latter are sometimes formed by the amœbæ following directly continuous tissue. This view is strongly upheld by a number of careful observers who maintain that amœbæ may even at times wander across the abdominal cavity and attack the liver at the apex of the dome. The rather frequent occurrence of subdiaphragmatic abscesses in places where the liver is not covered by its capsule is used as an argument in favor of this hypothesis. I have seen a few intestines in which the extensive ulcerations and adhesions about the hepatic flexure were so clearly associated with the abscess as to suggest direct infection. Other examples showing the disposition to wander which the organisms possess, are seen in the occasional amœbic pulmonary abscesses and empyemata which occur without liver abscess or other abdominal lesions. However, even after all these rarer occurrences have been taken into account there still remains the great majority of abscesses the presence of which can only satisfactorily be explained by assuming the portal venous system to be the channel of transmission, and, when we consider the histologic findings which are derived from a study of the intestinal infection, it is surprising that liver abscesses are not more frequent. Amœbæ, at times in great numbers, may frequently be seen in the blood vessels contiguous to the intestinal ulcers; in fact, where the lesions are extensive this may constantly be observed. These organisms may also be found in blood vessels in the intermuscular septa

and in other situations at considerable distances from the macroscopic lesions. In addition, amœbæ have been reported as occurring in emboli in the portal veins, and since this fact was first pointed out by Councilman and Lafleur and other observers, I have found it to be true in two instances, and in neither of these was there abscess or other indication of gross amœbic infection of the liver. Therefore, the evidence seems to be conclusive that in the majority of cases of well-advanced intestinal infection, amœbæ must almost constantly be carried to the liver, and this is particularly true in respect to those in which the thrombosis and endarteritis are modified by secondary invaders. If this is so, then actual liver infection must, at least partially, depend upon changes in that organ itself. However, in accepting such a tentative conclusion, a few other considerations must be advanced. The first and most important of these, namely, the influence of environment or symbiosis on the pathogenic action of amœbæ, has already briefly been dealt with by Musgrave and Clegg.[4] From this work and since that communication, from experimental data which we have obtained with cultures of amœbæ, it seems fairly established that these organisms are amenable to changes of environment which at first they would not easily tolerate. When such a change has been made, they are brought back to the original symbiosis with an equal amount of difficulty. The power of an amœba to propogate under new conditions may, to a large extent, depend upon the similarity between its new environment and the former one and also possibly, although this has not been proved, upon its ability to produce lesions in the tissues. If this should be true of intestinal infections it might be equally so of those of the liver, and the occurrence of liver abscess would then depend, as it apparently does, not so much upon whether amœbæ reach the liver or not, but upon whether or not the liver environment had, by both previous and present sub-intoxication, caused by the intestinal environment (see Adami's articles), been brought to a condition approaching that of the bowel in which the amœbæ were acting. This question is an extremely important one because in its answer by the use of cultures lies the possibility of an explanation of the exact mode of action of amœbæ in producing pathologic lesions. For work of this class the liver is a much more suitable organ than the intestine, because of the large variety of bacteria and other unknown influences in the latter. At present all of my work being carried on in conjunction with M. T. Clegg, of this laboratory, and in St. Paul's Hospital is in the direction indicated above, but the results so far obtained do not justify further statements.

Mouton, working with cultures of amœbæ, opened up another approach to this subject by studying the action of various serums upon "amœba diastase."

[4] *Publications Biological Laboratory, Bureau of Government Laboratories, Manila* (1904), **18**, 1.

The specific cause of this type of liver abscess is *Amœba coli*, either acting alone or in association with certain other micro-organisms. These parasites are constantly present in the contents or walls of true amœbic abscesses and no apparent differences are found between the amœbæ encountered here and those seen in the intestine. They may be the only organisms to be observed in the abscess at any time, or both amœbæ and bacteria may be present throughout the course of its growth, or, again, bacteria may be associated with amœbæ in the early lesions only to disappear at a later period. Both amœbæ and bacteria are often in the end not found in the contents of abscesses in which they were present at the beginning. Frequently the bacteria produce alterations, particularly in the nature of the lesion, so that it ultimately may more closely resemble a bacterial abscess. The bacteria which are encountered are of several different varieties. *S. pyogenes aureus* and *B. coli* are the most frequent; streptococci, pneumococci, *B. pyocyaneus* and several others have occasionally been found.

Bacteria are much more commonly associated with amœbæ in multiple abscesses than they are in solitary ones. This fact has been pointed out by Rogers and others and these observations are confirmed by the findings in the Philippine Islands. Multiple abscesses, sterile according to culture and animal inoculation, are occasionally met with, but this occurrence is much less frequent than it is in the large, solitary ones. In considering the reasons for this condition, several circumstances must be taken into consideration. Multiple abscesses are usually smaller and of shorter duration than are the single ones, and it is probable that, were opportunity offered at an early stage for bacteriologic study of solitary abscesses, more of them would show bacteria. In fact, to judge from the behavior of amœbæ on artificial media, it would hardly seem as if these organisms would be able to reach the liver, free from bacteria. No doubt, multiple abscesses are usually transmitted through the portal vessels, and under these circumstances bacteria within and attached to the amœbæ have a better opportunity of living and of multiplying than they would in some of the cases in which they probably reach the liver by directly continuous tissue.

A determination of the time of development of liver abscesses with reference to the bowel infection is not possible in the majority of instances. This is largely due to the slow development and absence of early symptoms in both diseases, however, the evidence tends to show that abscess may develop at any time during the intestinal infection. The literature on liver abscess in dysentery contains references to abscesses which have developed from a few days' time after the onset of the dysentery to those in which the affection was first diagnosed twenty-one years after the beginning. These records are based upon clinical evidences as given by the diagnosis of both the disease of the colon and of the liver, and therefore they are absolutely valueless in determining the only

question worth considering, namely, the shortest and the usual time elapsing between the development of the intestinal infection and the formation of the abscess. It has already been shown in previous papers that the appearance of diarrhœa and of other dysenteric symptoms bears little relation to the actual duration of the intestinal disease, and similar conclusions are true of the liver infection. The course of the disease, as will presently be shown, is slow and the diagnosis of uncomplicated abscesses is not usually possible at an early period.

The course and termination of amœbic liver abscess are both most certainly influenced by the bacteria which may be present therein. Multiple abscesses showing combined amœbic and bacterial infection, develop quite rapidly, and they usually terminate without perforation in the death of the patient, with a septic temperature. The same type when it is of purely amœbic etiology, has a slower course, the toxæmia is not so great, and patients often die of intercurrent trouble, with or without perforation of the abscess. In large single abscesses the course is still more chronic than in the latter case, and here also bacterial infection influences the variation and the outcome. It is undoubtedly true that, at times, patients having large, single amœbic abscesses of the liver remain in fair general health for several years.

Amœbic abscesses in general terminate in several ways. Where bacteria are associated with amœbæ, death may result from sepsis. A general amyloid condition is sometimes encountered at autopsy, as a result of the chronic sub-intoxication often seen as a sequel of large abscesses. Single ones are sometimes arrested and encapsulated, and may only be discovered at autopsy, years afterwards, or again they may completely heal. However, perforation is the most common termination, particularly of single abscesses, or of cases in which there are only a few large ones present and this may take place either externally or into any of the contiguous structures. By far the most frequent perforations are into the right lung or pleural cavity or into both. However, empyema of the left side, or pulmonitis may also occur, and this must be borne in mind as a possible favorable termination of abscess in the left lobe of the liver. The diagnosis of abscess in this location is much more difficult than when it occurs in other situations and surgical intervention is less likely; therefore, a larger percentage of these abscesses ruptures than of those of the right lobe. Perforations also occur into the abdominal cavity, intestine, stomach, vena cava, pericardium, retroperitoneal tissues, psoas muscle, kidney, urinary bladder, gall bladder, or spleen. I saw one case in the Army and Navy Hospital, Hot Springs, Arkansas, with perforation into the right pleural cavity, right lung, and externally through the skin. This patient recovered.

Symptoms and diagnosis.—The clinical picture varies greatly. Sometimes a large abscess may develop but slight symptoms and the diagnosis is not always possible even by exploratory puncture; however, in the

majority of instances the clinical findings will justify a diagnosis, which may be confirmed by evacuating a portion of the contents of the abscess through an exploring needle. Experience teaches the physician to be conservative in making these diagnoses during life, because fever, enlargement of the liver, pain, and the other common symptoms and the usual results of blood examinations may be quite well marked without the presence of an abscess, and again, a fairly large abscess may show almost no symptoms, even after a most complete and careful examination.

Physical signs.—The liver usually shows some enlargement, particularly if the right lobe is involved, and Osler has pointed out that this enlargement is usually more upward than it is downward. However, this condition is not so important in the Tropics, where some enlargement of the liver in foreigners and others most liable to abscesses is of rather frequent occurrence—the so-called "tropical liver." However, not infrequently rather extensive liver abscesses are found post-mortem, without any appreciable enlargement of the organ.

Fever—remittent, intermittent, or continuous—is usually present, but sometimes the temperature remains normal throughout the course of the disease. Septic temperatures are probably always due to mixed infections of amœbæ and bacteria in the abscesses. Two cases without fever at autopsy revealed solitary sterile abscesses of the right lobe. The fever is not at all characteristic. Not only may it be of any type, or on the other hand entirely absent, but the exclusion of other etiologic agents to which its production may be due is not always practicable. I desire to call particular attention to the fever which may also be caused by non-suppurative trouble of the liver. This is often present, and with local inflammatory conditions and adhesions about the hepatic flexure it makes a very deceptive picture. Rigors and sweats are usual with septic cases.

The blood count.—The number of the leucocytes and, particularly, their differential count, is generally given an undue importance in the symptomatology of this disease in the Tropics. Neither of these are of very great value in diagnosis. The intestinal infection is often responsible for a leucocytosis, which, when the associated bacterial infection is such as to give rise to acute dysenteric symptoms, may even reach above 20,000 for days or weeks at a time. Sometimes, with but slight intestinal symptoms, a moderate leucocytosis may be due to localized inflammation about the hepatic or splenic flexures or the cæcum. Frequently, all the suspicious symptoms of liver abscess, including the leucocytosis, disappear permanently after a few saline purges and large quinine enemas.

The *pain* of amœbic abscess is rarely acute and is not so frequently referred to the shoulder as it is in other kinds of abscess or in that of mixed infection. In the solitary, sterile abscess the pain is more of the nature of a general soreness over the liver and tenderness is usually found on pressure over its lower border or over the gall bladder. When the

abscess is of the sub-diaphragmatic type, pain is usually increased by a change of position, particularly by lying on the left side or by standing. In one case this dull, sore pain on standing or lying on the left side was the only subjective symptom of a subdiaphragmatic abscess; and this symptom disappeared before the diagnosis was made as a result of the perforation by the abscess into the lung. The local pains are most pronounced in superficial abscesses accompanied by perihepatitis, and the radiating ones in deeper disturbances and in septic conditions. As has already been mentioned, intestinal infection with inflammation and adhesions about the upper flexure of the colon may, in these instances, be deceptive.

The respiratory system.—Where abscesses rupture into the lung or pleura disturbances occur in the respiratory system. This is also the case when there is inflammation between the liver and diaphragm, or between the diaphragmatic and parietal pleuræ, a condition which sometimes occurs without actual extension of the liver abscess into the chest. No difficulty will be found in diagnosing an amœbic empyema. The lung is frequently the seat of lesions caused by rupture of the liver abscess and the diagnosis can be made by finding the parasites in the sputum.

Jaundice of a marked type is rare in solitary abscesses, and not very frequent in multiple ones. A slight subicteric tinge is quite common, but this is equally so in the non-suppurative hepatitis often associated with intestinal diseases. Except in septic conditions, there are no *stomach* symptoms of importance. There may or may not be nausea and there rarely is vomiting. One very important point which has not been properly emphasized is the influence which liver abscesses frequently exert on the intestinal symptoms. An increase in the number of the bowel movements during the development of abscesses is, to say the least, sufficiently common to be suggestive, and the removal of the liver pus by operation is nearly always followed by a temporary improvement in the number and character of the bowel movements.

Summary.—The symptoms of amœbic liver abscess vary greatly and may entirely be absent for long periods of time—sometimes for years. The diagnosis of the disease depends more upon the general picture, after excluding other diseases, than upon any system of direct examination. In the Tropics, the symptoms of an amœbic liver abscess may largely be the result of other conditions and diseases present at the time, and therefore these symptoms sometimes exist without liver abscess. In all cases the diagnosis should be confirmed by exploratory puncture and it must not be forgotten that even by this means a large abscess may not be located after very persistent use of the exploring needle. The diagnosis during life or before perforation is sometimes impossible and this is particularly true when the abscess is situated in the smaller lobes of the liver and in other inaccessible places. Except to differentiate between a situation in the right or left lobe, exactly to locate the abscess is

rarely possible, although this may occasionally be done by close, persistent observation and study.

The prognosis in multiple, inoperable, and septic abscesses is bad. In the solitary abscess which can be located, it is very good when the patient is in the hands of a competent surgeon. The treatment is surgical.

Abscess formations in locations other than the liver are occasionally seen. They most commonly result from perforation and may occur anywhere in the region of the colon, from the rectum to the cæcum. Perhaps the most common location is near the cæcum where, if abscess can be diagnosed, operation offers good chances of recovery. These lesions vary greatly in size and often in their development involve important structures such as the peri-renal tissues, the psoas muscle, the appendix, the abdominal wall, and sometimes the kidney itself. Large, ischio-rectal abscesses may occasionally result from a perforation in the rectum and in one such case, which will be discussed below, a large quantity of amœbic pus was evacuated. Abcesses occurring along the descending colon may be of a dissecting type and cases may be seen at necropsy, where, owing to perforations, adhesions, and ulceration, the entire intestinal wall may have sloughed away leaving a continent channel partly formed by the abdominal wall and adjacent structures. In rare instances, small abscesses formed and held in place by adherent omentum may be encountered on the anterior surfaces of the colon. One found in the descending colon at autopsy contained about 20 cubic centimeters of pus. Abscesses have also been reported in the parotid gland, the floor of the mouth, the brain, etc.

GASTRO-INTESTINAL COMPLICATIONS are very frequent and the after effects of dysentery on the upper portion of the alimentary tract are still more important. However, in the Tropics, a variety of disorders and diseases of this system are so frequently met with that it is very difficult to determine just how specific the relation to amœbic infection is and just which of the various troubles are most dependent upon this specific parasitic infection. Amœbæ are rarely found in the alimentary tube above the ileo-cæcal valve, and it is probable that the majority of diseases encountered in this portion of the bowel are not in a direct way dependent upon the amœbæ, but rather upon the associated dusturbing factors which so frequently attack the colon itself. In other words, the amœbic infection is usually a compound one and some of the symptoms in the colon and the majority of those above that portion of the bowel are due to agents other than the amœbæ. Most of the gastro-intestinal complications of amœbiasis develop rather late in the disease and may more properly be considered as sequelæ, or after effects, but some should also be mentioned here.

Cancrum oris has frequently been pointed out as a complication in dysentery and it is occasionally seen in the amœbic variety; however, it is perhaps met with less frequently than in the catarrhal, Shiga, or

streptococci types. It is of course most often observed in the terminal dysenteries occurring in the course of Bright's disease, diabetes, etc. Formerly this complication was seen more often in the Philippine Islands. Its disappearance has been due largely to the better care and treatment it is possible to give patients now than it was formerly. It is not clear or probable that *Cancrum oris* is directly associated with a specific infectious colitis in any form; it appears rather to be more dependent upon constitutional conditions.

Stomatitis and esophagitis occur more frequently as after effects of the disease, but sometimes do exist during its earlier stages. Superficial ulceration of the mucosa is the usual type of both these conditions and they are apparently always associated with lesions in the stomach and small bowel. Clinically, these are very important complications because they often are present before the gastritis and enteritis are marked enough to indicate the general condition of the tract. The appearance of the stomatitis is always a signal for more careful treatment, or the general condition of sprue will be the sequel.

Stomach disorders are particularly frequent in Manila and naturally are often associated with amœbic disease in a casual manner. However, in addition to this, there is more frequent and undoubtedly mutually dependent association between the two conditions, particularly in the later stages of a prolonged amœbic infection. The stomach symptoms which most interest us here are those of gastritis, gastralgia, and gastric ulcer. Gastralgia is particularly frequent and may be present with but a mild amœbic infection. The different types of gastritis with achylia gastrica and hyperchlorhydria are seen and gastric ulcer is relatively more frequent than in other diseases. All these conditions, together with nausea and indigestion, are undoubtedly made worse by rectal irrigations. In gastric ulcer it may occasionally be necessary, if indeed not always wise, to suspend large colon irrigations. In two cases of this combination recently studied in St. Paul's Hospital, gastric hemorrhages ceased with suspension of the colon irrigation which was being employed in the treatment of the dysentery. Hyperchlorhydria is, as a rule, also much aggravated by colon lavage.

Enteritis occurs with about the same regularity and in the same class of patients as does gastritis. In addition to the stomach troubles, it is one of the most important complications of the disease because of the ultimate results due to lack of assimilation of food. Reversed peristalsis from local treatment of the dysentery is not uncommon and often very annoying.

Renal complications, and particularly hæmorrhoids, are frequent, while fissures and fistulas occur less often. Hæmorrhoids are usually aggravated by treatment, and in some instances seem to owe their origin to it.

Complications of the large bowel are perhaps the most interesting and frequent, and the least written about. Particularly may be mentioned

dilations, contractions, and cicatricial formations of the bowel; other types of infection and of dysentery, such as the specific bacterial, gangrenous, diphtheritic, catarrhal, and miscellaneous types; and those terminal ones to be seen in nephritis and other constitutional diseases.

Dilation of parts of the colon are frequently encountered and almost general dilation is occasionally observed post-mortem. The latter condition may also be diagnosed during life. Whether they are either general or local, dilations are made possible by the relaxation of the circular and longitudinal muscular bands of the intestine. In advanced cases the bowel takes on the appearance of a smooth tube with partial or complete absence of the rugæ and folds. The picture is rather that of a post-dysenteric process than that of a complication during the active stages of the disease. However, it is also seen as a complication at times. One patient who died recently in St. Paul's Hospital showed this condition in a marked degree. The amœbic disease was probably of several years' duration, and death was due to an acute streptococcal dysentery lasting only a few days. At autopsy, the width of the opened bowel measured on an average 15 centimeters, and in the cæcum it reached 20 centimeters. The clinical manifestations of such dilation are interesting. When it occurs early in the disease it is usually, if not always, associated with troublesome gas collections in the bowel and a tendency to constipation, but as an after effect, the sprue diarrhœa is its constant associate. Deep, undermined ulceration is rarely found in an intestine of this nature and it does not appear, as might be supposed, that intestinal irrigation has to do with its production.

Cicatricial contraction from healed amœbic ulcerations is observed less frequently in the Philippine Islands than it is stated to be in other parts of the world. It is occasionally seen in a moderate degree, and sometimes to such an extent as to cripple the bowel function, but on the whole it is of but little importance as a complication in this country.

Other dysenteries and bowel diseases complicate amœbiasis with great frequency and have not received the careful study which their importance merits. The acute bacterial dysentery (Shiga) has not been very frequently encountered in the Philippine Islands for some years, but there have been undoubted cases where it has complicated the amœbic infection and others where it has preceded or followed such disease. The combination is an exceedingly difficult one to treat; the pathologic picture is modified and the diagnosis difficult on account of the tedious methods necessary for the isolation of *B. dysenteriæ* from the stools. The serum reaction is not of very great clinical value. The bacteriology of all of the acute dysenteries is not known, but there surely are organisms, in addition to Shiga's bacillus, which under proper conditions play an important etiological role. It is also likely that this group is greater in the Tropics than in the temperate zones, and also possible in these regions that there is an increased virulence of some of the well-known

organisms, perhaps caused by their changed environment, and also a decreased resistance on the part of the patient. The possibility of the influence of symbiosis with animal parasites on the virulence of such organisms as the colon bacillus needs to be elucidated. Whatever the etiologic factors may be, it is a fact that the engrafting of acute dysentery on an old amœbic process is of quite frequent occurrence in the Philippine Islands. The character of the amœbic lesion is too far removed from those seen in such conditions as to admit of any other interpretation than a double etiology. Diphtheritis, gangrene, folliculitis, etc., are common complications and bear little or no relation to amœbic etiology. The ulcerations in the terminal dysenteries in nephritis, diabetes, splenomegalies, cirrhosis of the liver, and other diseases may, as pointed out in previous publications, complicate amœbiasis and alter the clinical picture, pathology, and prognosis very materially.

Finally, there remains to be mentioned here the association of intestinal parasites, such as paramæcia, strongyloides, oxyuris, trichomonas, circomonas, megastoma, tænia, trichuris, ascaris, and others with amœbæ. Some of these parasites, particularly *Balantidium coli* and *Strongyloides intestinalis,* are now quite generally acknowledged to be pathogenic parasites, but many others are likewise considered to be harmless. However, I believe the almost unanimous concensus of opinion of the careful, experienced workers in Manila is voiced in the statement that some of these so-called harmless parasites are disease producers. For example, many men here recognize a diarrhœa caused by monads. Several types of these parasites, when present in large numbers, are very intimately connected with chronic diarrhœa and they are surely much more important than they are generally considered to be.

Megostoma entericum (*Lamblia intestinalis*) deserves particular notice. It is often found and, when encountered in great numbers, it is *always* associated with chronic diarrhœa, which disappears with the destruction of the parasites. In my opinion, this flagellate bears a decided causative relation to the diarrhœa. Even the *Trichuris trichuria* certainly produces small, hæmorrhagic spots where it adheres to the mucosa of the cæcum and some of these, as in a case now being studied, are closely associated with very early amœbic lesions. Whatever pathological significance may be attached to these parasites in general, some of them, particularly the actively motile ones, such as monads, surely aggravate amœbic ulcers in which they may be present.

Perforation of intestinal ulcers is one of the very frequent complications of untreated and of fatal cases, but is comparatively rare when proper therapy has been instituted early and followed persistently. These perforations may occur in various places and give rise to acute general peritonitis, localized peritonitis, or abscess formations.

Strangulation of either the large or small intestine may occur from several causes. Partial or complete strangulation from external pressure

by fibrous bands following localized peritonitis deserves special attention because it is not so infrequent and because it has previously received but slight consideration. Rogers mentions a case of strangulation of the small intestine from such a cause, and I have observed several cases, post-mortem, in which such bands were placed in a manner in which they must materially have interfered with the function of the organ. These conditions may exist in any portion of the colon, but they are most common below the splenic flexure; one case has been encountered where an hourglass contraction of the cæcum was due to such a cause. The omentum is a factor in the majority of these cases because it is the important agent in the localized peritonitis which is very frequent in the disease. Many of the fibrous bands are formed principally about the omental-intestinal adhesions. Except in very rare instances, these contractions do not produce results which contribute materially toward a fatal termination of the disease, but, on the other hand, they are the cause of certain symptoms, both during the course of the disease and afterwards, in patients who recover. The symptoms usually are not of such a nature as to allow of a definite diagnosis of the condition, nor are they of very great importance, excepting where some other condition is to be excluded.

Intestinal hæmorrhage is a comparatively rare complication of amœbic infection, as is to be expected from the histologic picture which is produced, such as the well-known thrombosed condition of the blood vessels within and closely surrounding the lesions, and the more or less well-marked endarteritis which is usually present. Hæmorrhage has been noted in a general way by several writers, but its importance as a factor to be reckoned with in the prognosis and treatment of the disease has not, until recently, received very much consideration. Attention had been called to it, in dysentery, by Woodward, Scheube, Manson, Sodre, Osler, Lafleur, Ranaud, and others, but its special importance in relation to amœbic dysentery has been emphasized by Lafleur, Haasler and Strong. The association of these hæmorrhages with liver abscess has also been noted by Woodward, Haasler, Strong, and Mugliston and Freer.

Haasler[5] briefly reported three cases of severe intestinal hæmorrhage in amœbic dysentery, in two of which the hæmorrhage was considered to be the cause of death. The author notes particularly that in both of these cases there was liver abscess and in one, with death after a loss of over 4 liters of blood, a thrombosed vessel was found in one of the ulcers. Strong[6] again emphasized the occurrence of severe intestinal hæmorrhage as a fatal complication of amœbic dysentery, and reported to the Manila Medical Society November 2, 1902, two cases of amœbic dysentery and liver abscess in which severe hæmorrhage was the cause of death. One of the patients lost more than 2 liters of blood in repeated hæmorrhages during the three days preceding the end. At necropsy the source of hæmorrhage was

[5] Haasler: *Deutsche Medicin. Wochenschr.* (1902), **28**, 26 and 47.

[6] Strong: *Publications Biological Laboratory, Bureau of Government Laboratories* (1905), **32**, 1.

found in a thrombosed vessel occurring in an ulcer situated about 5 centimeters below the cæcum. In his other case the point of hæmorrhage was not located at autopsy. He also later reported two more similar cases, again emphasizing the presence of liver abscess. In one of these he called attention to the delayed coagulation time of the blood.

Mugliston and Freer[7] report two cases of amœbiasis with abscess of the liver and fatal intestinal hæmorrhage. Both of these had septic temperatures and in one, jaundice during life was noted.

I have seen one fatal and four other cases of severe intestinal hæmorrhage in amœbiasis without liver abscess. In the fatal case, a native prisoner, the amœbiasis was associated with Banti's disease and the hæmorrhage was both gastric and intestinal. One patient, with two hæmorrhages aggregating about 1,200 cubic centimeters of blood in twenty-four hours, also had an amœbic, ischio-rectal abscess. In one, with repeated small hæmorrhages, there was also tuberculosis of the bowel and in the remaining two there was some severe secondary invader in the intestine, as was shown by the severity of the symptoms, which included fever, tenesmus and depression, conditions which probably do not occur in uncomplicated amœbic infections.

Strong discusses the relations between liver abscess and severe fatal hæmorrhages and refers first, to the question of whether the destruction of such large amounts of liver tissue may not sometimes bring about serious functional disturbances in this organ and lead to a condition which predisposes to hæmorrhage; second, to the mechanical interference caused to the portal circulation; third, to the relation between hæmorrhage and various other diseases of the liver, particularly when jaundice is present; fourth, to the diminished coagulability of the blood; and fifth, to special bacterial (toxic) activity. He also suggests the occasional possible diagnostic importance of such hæmorrhages in liver abscesses.

I am of the opinion that the association between amœbic liver abscess and hæmorrhage is because of an interdependence upon a common condition which is discussed more fully under abscess of the liver. It will be noted, in looking over the literature of intestinal hæmorrhage in dysentery, where the records are specific there is often evidence of severe infection which can not be attributed to amœbæ, and this is particularly true in the cases associated with liver abscess. Strong's and Mugliston's and Freer's cases had slight jaundice; there was fever and other evidences not found in amœbic infection without the presence of some other etiologic agent and which, by their action, might bring about the general conditions which favor intestinal hæmorrhage in other diseases. In discussing the etiology of these hæmorrhages we must not lose sight of the condition of the intestine, particularly with reference to the nature of the secondary invaders which may be present. The majority of the cases which have been reported were in infections of a gangrenous or

[7] Mugliston and Freer: *Jour. Trop. Med.* (1905), 8, 113.

diphtheritic or other very severe type, the lesions in which were clearly not due to the action of the amœbæ. We know that such agents may alter the whole type of an amœbic lesion and bring it histologically nearer those in which bleeding is a more common occurrence.

The symptomatology and treatment of intestinal hæmorrhage caused by amœbic ulceration is the same as when it is due to other causes and need not be entered into here.

Sloughing of the mucosa is preëminently a complication of this disease and in untreated cases is fairly frequent. The sloughs may be of any size, from small shreds to casts of the bowel several centimeters in length. Sometimes at autopsy, considerable areas are seen completely denuded of mucosa.

AMŒBIC APPENDICITIS.—Involvement of the appendix in amœbic infection of the colon has been noted by several writers. It has already become a subject of considerable importance in the Philippine Islands, and the larger number of patients returning to the United States from Manila and other tropical countries makes it of considerable importance to the profession at home. In my first report[8] of 150 postmortem examinations of fatal cases of intestinal amœbiasis, 100 of which were from the records of Strong and myself, 6 per cent showed amœbic lesions in the appendix and in 7 per cent more there were other lesions of this organ and 1 case had amœbic ulceration engrafted on an old, chronic appendicitis of other etiology. In a second series of 100 fatal cases reported by Woolley and Musgrave[8] there were 8 with amœbic lesions in the appendix and in all, as in the first series, the lesions appeared as a continuation of an amœbic process in the cæcum; in the last series they also appeared in the small intestine, just above the ileo-cæcal valve. The above and many additional pathological data which have been obtained, together with a large amount of clinical material, is the basis for the present consideration of the subject.

Etiology.—The disease is much less frequently met with at autopsy than one would suppose, judging from clinical observation. It was formerly more common than it is now in the Philippine Islands. This decrease is largely due to the more general early diagnosis and successful treatment of the colon infection at the present time. It is nearly or quite always an extension of the amœbic process from the cæcum and shows a specially marked relation to infection on and above the Bauhinian valve. It probably does not often develop early in the disease, for it was not present in a single one of 25 early cases reported by Woolley and myself,[8] in which death was caused by intercurrent diseases. It most often attacks an otherwise normal appendix and but rarely one already crippled by former disease, and it is very much more frequent in severe dysenteric infection, with intercurrent diphtheritis, gangrene, etc.

[8] *Loc. cit.*

Morbid anatomy.—The lesions of the amœbic process in the appendix resemble those found in the bowel, but they are usually smaller and more superficial. Microscopically, the same general picture including thrombosis of the vessels, is seen. As in the colon, the ulcers are rarely along the meso-border but they sometimes may extend entirely around the mucosa. The appendix is most often patulous, with a rather large lumen, and concretions are exceptional. External evidences of inflammation are not very marked; as a rule, they consist of moderate engorgement and injection of the vessels.

Symptoms and diagnosis.—There is not very much to add to the general symptomatology as given in a previous communication.[9] The clinical manifestations in general resemble those in appendicitis from other causes, but are as a rule less severe. There is less rigidity of the abdominal muscles, the tumor mass is not so sharply circumscribed, and the fever and general prostration are less marked. However, cases may be of a fulminating type and are then not to be distinguished clinically from other types of appendicitis, but then these, like very acute intestinal symptoms, are due more to secondary invaders than to the amœbic infection. Appendicitis, in no way caused by amœbæ, may occur in a patient suffering from amœbic dysentery. The leucocyte count is not of as much value in amœbic appendicitis as it is in other types, because the intestinal infection plays too uncertain a part in the variation of the number of the white cells. The following pathologic changes found in intestinal amœbiasis may give clinical symptoms closely resembling appendicitis and must be taken into account in a consideration of the subject:

(1) Severe amœbic infection of the colon or cæcum and ascending colon.

(2) Infection of the lower ileum with the cæcum.

(3) Acute, localized peritonitis about the cæcum, ascending colon, or hepatic flexure.

(4) Pericæcal amœbic abscesses.

(5) Neuralgia, gaseous distention, and fæcal accumulation in the cæcum.

(6) Acute catarrhal appendicitis.

(7) Chronic recurrent appendicitis.

(8) Chronic recurrent appendicitis with secondary amœbic infection.

(9) Amœbic appendicitis.

(10) Any combination of the above conditions.

The first of these conditions is a *very* frequent one in Manila and superficially at least, it closely resembles appendicitis. The differentiation, however, is not, as a rule, difficult upon careful clinical examination by one familiar with all the conditions. The fever, leucocyte count, nausea, and

[9] *Loc. cit.*

vomiting (particularly if the patient is taking enemas), abdominal tenderness, etc., may indicate appendicial involvement, but the history of the case, careful palpation and percussion, lessened or absent rigidity of the abdominal muscles, the area and diffuseness of the tenderness and the location and character of the tumor mass, make the diagnosis fairly easy in the majority of instances. This is the class of cases where frequent and needless appendicectomies are done by some physicians with more surgical ambition than judgment. Having performed the autopsies on a few of these, examined the removed appendices in several, and later treated the condition of the bowel in a number of others, I can not condemn this practice too severely. The operation in this class of cases is not only useless but dangerous, because of the delay which it causes in the treatment of the condition of the bowel.

Cases which may be classified under the second, third, and fourth headings very closely resemble, in clinical symptoms, the class just described and two or more of the conditions are, in fact, often combined. It is not possible to diagnose amœbic disease of the lower ileum with certainty. Acute, localized peritonitis and other adhesions about the cæcum frequently occur, particularly in severe infections and are usually quite painful. The omentum is often plastered about the cæcum and adjacent structures and, when the bowel is empty and there is not too much tenderness, the condition should be recognized by the experienced examiner. Pericæcal amœbic abscesses are usually recognized by the same manifestations as are those from other causes; they have a strong tendency to spread into the surrounding tissues.

Gaseous distention and even fæcal accumulations in the cæcum are frequently met with in patients taking enemas and often give rise to a painful condition. The symptoms may be relieved by an active cathartic, which should also be used before a final diagnosis of other conditions in the region of the cæcum is attempted. Severe symptoms are often permanently relieved by one or two doses of salts.

Acute, catarrhal appendicitis may, of course, occur during the time a patient is suffering from intestinal amœbiasis. This complication, curiously enough, is comparatively rare, but when it is the case the symptomatology and treatment are the same as in appendicitis occurring in an otherwise healthy person.

Chronic recurrent appendicitis is no more or no less frequent in this disease than in other classes of patients. Recurrences in appendices where amœbic infection is present are sometimes due to close association with the diseased cæcum. An amœbic infection is sometimes engrafted upon such an appendix, but this is of very rare occurrence and the diagnosis is then not only difficult but often impossible.

Primary appendicitis is the most frequent type of amœbic infection of the appendix. Its clinical manifestations, when there is an occlusion, closely resemble acute appendicitis from other causes. These infections

are almost always accompanied by an extension of a severe diphtheritis or other secondary involvement of the cæcum. There are a number of cases of a very mild type of undoubted amœbic ulceration of the appendix, accompanied by the discharge of material through the patent lumen, with recovery under medical treatment. Unless there is some severe secondary invader, the amœbic lesions are particularly apt to be near the cæcum, and, when more distant, are less likely to produce serious trouble than in any other type of appendicitis. All varieties of combinations of the ten headings given may be encountered, and to deal successfully with them requires a close study of the individual cases and a comprehensive knowledge of the subject.

Prognosis and treatment.—If proper treatment is given, the prognosis is usually good in nearly all the conditions mentioned above, and it is particularly so when the appendix itself is not actually involved in an acute catarrhal or amœbic infection. However, in quite a number of patients when the symptoms which have been detailed supervene in a case complicated by very severe diphtheritic or gangrenous cæcum or appendix, the outlook is bad, regardless of the treatment. The treatment of most of these conditions is medical, under close surgical consultation. Large, high enemas combined with saline cathartics and local applications permanently relieve most of these patients. When the appendix is really involved, although many of these cases undoubtedly get well under medical measures, the treatment should be surgical, but of a conservative nature. Great care in diagnosis should be used and unnecessary surgical intervention carefully guarded against, because the operation of necessity delays treatment of the dysentery. This may prove a very serious matter and in some instances in Manila has surely been responsible for the fatal outcome. The operation of appendocostomy or cæcostomy, as advocated by Tuttle and others, might occasionally serve a double purpose in these patients. This operation has been performed several times in Dr. McDill's surgical service in St. Paul's Hospital and the cases will be fully reported by him.

ANATOMIC DISPLACEMENTS AND MALFORMATIONS, such as ptosis, unusually long mesentery, diverticula, etc., may be of serious enough import to be classed as complications. Enteroptosis, consisting of a V-shaped descent of the transverse colon which consequently forms a sharper angle at the hepatic and splenic flexures of the colon, may become of particular importance, due largely to the proneness which these two points have to severe amœbic involvement and the difficulty of securing satisfactory irrigation of such a bowel. Such positions of the transverse colon are not infrequently seen at autopsy and are to be at least partially, explained either by the increased weight of this portion of the intestine due to inflammation or to adhesions between the intestine and omentum and some portion of the abdominal wall. Unusually long mesenteries and peculiar flexures in the sigmoid and rectum deserve to be mentioned because of their importance in the introduction of rectal tubes.

ACUTE, GENERAL PERITONITIS usually follows perforation of an ulcer in the colon, or of a liver abscess, but this complication does occur without any perforation whatever and, on the other hand, perforations of the ulcers in the colon may be walled off without general peritonitis. In rare instances, the rupture of a liver abscess into the peritoneal cavity may take place without producing general peritonitis. In 100 carefully examined, fatal cases of amœbiasis, acute general peritonitis occurred 26 times; 4 times from ruptured liver abscesses; 20 times from perforation in the large bowel; once from a perforated appendix, and once without any perforation. Of the colon perforations, 8 were in the sigmoid flexure and descending colon; 2 in the transverse colon and 9 in the ascending colon and cæcum. In the case of peritonitis without perforation there was a combination of acute and amœbic dysentery. These statistics are, of course, from extreme cases and in no way represent the prevalence of peritonitis in any other class. It is comparatively a rare occurrence in patients who are satisfactorily treated and even in an average series of fatal cases it will be found much less frequently than it is in the statistics quoted above. The symptomatology and treatment of this complication needs no comment here.

CHRONIC, LOCALIZED ADHESIVE PERITONITIS is one of the most frequent complications of the amœbic infection and is found in more than 80 per cent of the fatal cases. It may be very slight or very extensive and may give rise to all varieties of adhesions which may occur in the abdominal cavity. Some of the most important of these adhesions are of the omentum to the various other adjacent structures, such as to the abdominal wall, the surfaces of the intestines, the appendix, and particularly to the cæcum; of the surfaces of the intestine to each other, to the abdominal wall, the appendix, stomach, gall-bladder, liver, spleen, etc.; and the adhesions between the liver and diaphragm, abdominal wall, colon, and even appendix. Many other varieties and combinations are encountered. The principal symptoms of these various conditions are abdominal pain and soreness. They may be so slight as not to be noticed, and, again, may be overshadowed by pains of other origin during the active stages of the intestinal disease. The most annoying manifestation of these adhesions develop as an after effect in this disease, and these conditions will be further considered when that part of the subject is discussed.

PHLEBITIS, EMBOLI, VENUS THROMBOSIS, AND INFARCTIONS have been reported and infarcts in the liver, spleen, and intestine are sometimes seen. Within the last few days multiple, white infarcts of both kidneys were found at autopsy in a case of intestinal amœbiasis combined with mesenteric and intestinal tuberculosis.

PERNICIOUS ANÆMIA has but rarely been noted as a complication in amœbic disease. I have seen two cases in which the association was intimate and an apparent interdependence continued during the course of the disease. Both were patients who had contracted their maladies in

the Philippine Islands and came under my care in the Army and Navy Hospital at Hot Springs, Arkansas. It was impossible to determine which disease antedated the other, but the clinical evidence of the double infection was conclusive. The particularly interesting feature in both of these cases was the parallelism between the clinical manifestations and the blood changes and the bowel symptoms. Temporary improvement in the dysentery was also closely followed by an improvement in the anæmia.

A CASE OF LEUKÆMIA AND AMŒBIC DYSENTERY.—One may be mentioned in connection with these two instances:

This case, like each of the former two, occurred in a discharged volunteer American soldier who had served in the Spanish-American war in Cuba and in the Philippine Islands, and who came under my observation during my service in the Army and Navy Hospital at Hot Springs, Arkansas. The apparent interdependence of the two diseases in this instance was most striking and was studied and confirmed by the late Major Walter Reed, to whom the patient was referred. The dysentery was undoubtedly contracted in Cuba, and enlargement of the spleen was first noticed about eighteen months later, while the patient was serving in the Philippine Islands. The history of the dysentery was the usual one of alternating diarrhœa and constipation, abdominal soreness, and loss of weight. The enlargement of the spleen was progressive. When he came under observation there was moderate emaciation, great weakness, shortness of breath, and slight jaundice. The dysentery was very active. There were from ten to fifteen stools per day, associated with some blood and mucus, and numerous amœbæ. The spleen was very large, extending to the pelvic brim below and beyond the median line to the right. A blood count showed splenic leukæmia with 460,000 white cells. As was mentioned when the two cases of primary anæmia were discussed, the interdependence in this case between the amœbic and leukæmic diseases was decidedly marked, and improvement or exacerbation of one disease was followed by a like procedure in the other. This same general feature has been noticed recently in the study of *splenomegaly*. There have occurred a number of cases of a combination of amœbiasis with splenomegaly, and the association sometimes seems a close one.

CHRONIC RHEUMATISM, both of the articular and muscular types, is very frequently met with in Manila and in many instances seems to bear a very definite relation to amœbiasis as well as to other severe disturbances of the stomach and bowel. Even where alcohol and other recognized causative factors have been eliminated, we still have a frequent association of the two diseases which is hard to explain without acknowledging some form of interdependence. The rheumatic condition is particularly apt to develop in cases of amœbiasis of long standing, and it often shows clinical intensity corresponding to and varying with the condition of the bowel. In other instances, an old rheumatic condition is rendered much worse by an intercurrent dysentery. The clinical evidence is very strongly in favor of dysentery being in some way responsible for one variety of the symptom complex termed chronic rheumatism.

THE NERVOUS SYSTEM, as in the case of chronic rheumatism, often shows disturbances intimately associated with amœbic dysentery. Allen

Starr and other eminent neurologists have pointed out the close association of certain forms of neuritis and neurosis with disturbances of the gastro-intestinal tract, and to these must be added amœbic infection of the colon. Many old, nervous troubles, particularly *sciatica*, are excited by amœbic dysentery, and, in addition to this, the beginnings of various chronic types of nervous disease may be traced to the date of a previous dysentery.

Acute chorea.—There are in the literature a few references to choreic movements complicating this disease, but, so far, no reference to Sydenham's chorea complicating dysentery of any kind has been found. I have seen two instances in which the association of true chorea and the amœbic infection of the colon was very close. Briefly, these cases are as follows:

CASE I. *Intestinal amœbiasis; rather severe infection; acute chorea; recovery; recurrence of both dysentery and chorea; recovery; typhoid fever without chorea; permanent restoration to health.*

Miss X, American, 23 years of age, in Philippine Islands for eighteen months. No chorea in family and no previous history of choreic movements. No history of rheumatism, heart affection, or other disease or tendency to chorea. No previous illness of any kind. After about two weeks of indigestion with intermittent diarrhœa she was taken suddenly during the early evening with acute abdominal pain and nausea. By next morning the bowel movements showed mucus and blood; there was about 1 degree of fever, and tenesmus and nausea were present; microscopically, the fæces contained numerous amœbæ; there was much blood and mucus, sloughs and shreds of mucosa of the bowel. She was treated during the first forty-eight hours with cleansing enemas, mild saline laxatives, and the pain controlled by Dover's powders. On the third day acute symptoms had so much subsided that she was placed on quinine enemas twice daily. The fourth and fifth days were comfortable, no fever, no nausea, but still considerable abdominal pain and rather frequent bowel movements. On the morning of the sixth day choreic movements of the hands were noticed, and by the next the movements were general, including those of the face and legs. There was decided hesitancy in speech; on the tenth day pain and slight swelling of both ankles developed, continued for about twenty days, and gradually disappeared. There was, for some days, considerable tachycardia, the pulse counting from 90 to 105 per minute. The rhythm was a little irregular and a soft, systolic murmur was heard at the apex. There was no evidence of enlargement of the heart, and the murmur disappeared during convalescence. I do not believe there were organic changes in the heart. The chorea and dysentery subsided together during the next three weeks. About six weeks after the onset of the dysentery there was a moderate recurrence of this symptom, although treatment had been continued during this time. With the recurrence of the dysentery, there was return of slight choreic symptoms, confined to the hands and face, and lasting only a few days. Recovery from both the amœbic infection and chorea was permanent. About six months later this patient passed through an attack of typhoid fever. No chorea was seen during this illness, and she has been entirely free from it up to the present time, two years since the dysentery.

CASE II. *Neurotic young woman, 22 years of age. Chorea in childhood, with rheumatism following scarlet fever. Amœbiasis, severe. Chorea; recovery.*

The interesting factors of this case were the relation of the attacks of dysentery to those of the chorea which developed together. The chorea subsided about ten days after the bowel movements had become normal.

In both of these cases the intimate relationship between the dysentery and the chorea was such as strongly to indicate that something was being absorbed from the diseased bowel which caused or aggravated the chorea. When we remember the close association of chorea and rheumatism, and, as just mentioned, that there is also a rather close relation between dysentery and certain cases of rheumatism, the instances described above appear in a more natural light. In both the cases treatment was directed to a cure of the dysentery. In the first one the enemas were stopped for a few days, as it was thought they might have something to do with the trouble, but their interruption was without influence on the character or intensity of the nervous disease.

ALBUMINURIA is very common in long-standing cases of amœbiasis, and organic changes undoubtedly frequently exist as late complications and after effects. However, excepting the association of the two diseases (which has already been discussed) where the kidney trouble is clearly of other etiology, *permanent* impairment of the kidney function does not appear to be a serious outcome of the amœbic disease.

Among the other complications of amœbiasis, which have from time to time been reported, are mentioned fibroid degeneration of the heart, terminal pericarditis, purpura, gangrenous ulcers of the stomach, mastoid abscess, abscess of the brain (Kartulis), pseudoparalysis, gangrene of the foot, etc.

IV.

AFTER EFFECTS.

The degree of restoration of function which will occur after the most extensive amœbic ulceration of the colon has been repeatedly pointed out. In the majority of well-treated cases of simple amœbic colitis there are no serious sequelæ, and even in some of long standing, with the passage of blood and mucus and even of sloughs, regeneration may be so satisfactory that perfect function of the bowel may be restored. However, sequelæ are not rare in amœbiasis, and, while not of so great a variety as the complications, are nevertheless of great importance. Like the complications, they divide themselves into sequelæ directly due to the amœbic infection, those due to the complications of the disease, and those but remotely connected with the amœbic disease. This latter group is a somewhat large one, including constitutional disturbances (conditions arising as a result of wasting, lowered resistance, etc.), and it need not here be entered into in detail. There are several important conditions of the other two groups to be discussed of which the first and one of the most frequent is sprue.

Sprue.—The superficial, clinical picture and the anatomic findings in sprue are fairly constant; enough so, perhaps, to justify its consideration as an entity, but the etiology is surely multiple, *and therefore, at least etiologically, it must be regarded as a symptom complex and not as*

a distinct disease. I have already called attention to this fact in previous publications, and several additional years' experience have confirmed the first observations. This condition is fairly common in the Philippine Islands and is becoming more so as the years go by and we follow our old, chronic, gastro-intestinal cases to later stages and the autopsy table. It is a clinical picture arising early or late in a variety of wasting diseases, particularly in those actively attacking the gastro-intestinal tract. However, this condition only interests us in this paper in its connection with amœbic disease, which, however, includes the majority of cases encountered in the Philippine Islands. It may appear somewhat early, but more usually late in the amœbiasis and often long after all evidences of the amœbic infection have disappeared. It is particularly liable to develop in those cases of amœbiasis complicated by disturbances of the stomach and small bowel, which have already been discussed. The symptomatology is so simple and has been so frequently described that it need not be noticed here. The salient points in the pathology were given by Woolley and Musgrave. A more complete study of both symptomatology and pathology would lead to a consideration of the diseases concerned in its multiple etiology; such a consideration has always been avoided by those classing it as a distinct disease. It is one of the most intractible conditions to treat which is found in the Tropics. This might be expected when we remember the principal pathologic changes found at autopsy, particularly the enlarged, thin-walled intestine, with atrophied cystic and often papiloid condition of the mucosa of the whole gastro-intestinal tube and the relaxation and partial destruction of the muscular bands of the bowel, which leave it elongated (often increased in length to 2–4 meters), enlarged in diameter, and without its natural folds and rugæ. Change of climate is necessary in most well-established cases in order to secure satisfactory results in treatment. Chronic gastro-enteritis does not of course always give rise to symptoms of sprue. This depends somewhat upon the nature of the etiologic factor and more upon the character of the fermenting bacteria, yeasts, and other substances multiplying in the bowel.

Abdominal pain and soreness persisting for years after amœbiasis, is quite a common sequel, and this condition is particularly frequent in patients who have had severe and prolonged attacks. It is undoubtedly due to areas of chronic adhesive peritonitis formed during the active stages of the disease. The symptomatology varies with the character and location of the adhesions. The clinical features may resemble a chronic appendicitis, when at autopsy none is found. Pains in the region of the liver, spleen, stomach, ovaries, and many other locations are met with. By far the most common character of the pain is one of general abdominal soreness, which is increased when the patient is in the erect posture and is relieved by his lying down. Just the reverse of this is also seen, in

which patients are never comfortable in the recumbent position. These pains may last for years after the disappearance of the disease or they may recur, particularly after exposure to cold or after violent exercise. The writer was, for more than a year after recovery from amœbic infection, troubled with these pains, and even now, more than five years since the original disease, a chilling of the abdomen, a slight diarrhœa, or an active cathartic will cause a recurrence of some pain. Treatment of the condition is not, as a rule, satisfactory. Carefully regulated exercise and massage may hasten the adjustment to new conditions and, sometimes with splanchnoptosis, proper bandages and corsets might advance a favorable result.

A considerable number of diseases and conditions already discussed under complications, particularly those appearing late in the disease, show their most decided results as after effects of amœbiasis. Among these are different types of gastro-intestinal disturbances in addition to sprue, chronic rheumatism, various disturbances of the nervous system, genito-urinary system, skin, etc. Therefore, the after picture is a very varied one; complete restoration to health is the most frequent outcome, but many of these cases go through life crippled by every complication which may be seen in other chronic, wasting diseases. The patients for the greater part disappear from the Tropics, but an extensive visit throughout the United States and careful scrutiny of our increasing pension roll will show us some of the awful ravages which this worst of all parasitic diseases is making on the American colonizers of the Tropics.[10]

[10] The inadvertent omission of the discussion of amœbic infection of the urinary bladder from this article has only been noticed in reading the proof. This subject was discussed by McDill and Musgrave in *Medical News*, December 16, 1905, page 1163. It will shortly again be taken up with the report of six more cases.

REVIEWS.

A Vocabulary of Malay Medical Terms. By P. N. Gerrard, B. A., BCH., B. A. O., M. D., Dub. Univ., D. T. M. H., Univ. Cantab. Pp. 107, Singapore: Kelly & Walsh Limited. 1905.

This work is a well-written vocabulary of Malay medical phrases and terms. European and American physicians in the East have to depend almost entirely upon bedside diagnosis. The author has here presented definitions and interpretations in a concise form. The subjects of his conversations include some of the important diseases of the country, thus affording to physicians an easy method of procuring a clinical history, frequently a valuable asset in diagnosis.

M. T. C.